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OVARIAN FUNCTION FOLLOWING PITUITARY STALK-SECTION
OR HYPOPHYSECTOMY IN THE PIG

by

Gerald Wayne Dyck

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
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DOCTOR OF PHILOSOPHY

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Approved:

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1966

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INTRODUCTION

The function of the mammalian ovary is dependent upon the pituitary, central nervous system and the uterus. The investigations of Smith (1926, 1930) demonstrated that ovarian function in the rat was controlled by the anterior pituitary. Hypophysectomy resulted in ovarian regression, whereas injections of suspensions or extracts of pituitary tissue maintained ovarian function. Moore and Price (1932) demonstrated the presence of an interaction between the pituitary and the ovaries. Later, this interaction was described as "negative feedback" as follows: Gonadotropin secretion from the pituitary stimulated the growth of Graafian follicles in the ovaries. These follicles produced increasing quantities of gonadal hormones. The production of increasing quantities of gonadal hormones reduced the gonadotropin content of the pituitary, which in turn reduced gonadal hormone production by the ovaries. Hohlweg and Junkmann (1932) proposed that a steroid sensitive "nervous sexual center" in the hypothalamus mediated the effects of the sex steroids on anterior pituitary function. This mode of action was proposed to explain the absence of pituitary "castration" cells when this gland was separated from the hypothalamus.

The influence of the uterus on ovarian function was demonstrated by prolonged maintenance of corpora lutea in hysterectomized guinea pigs (Loeb; 1923, 1927). If corpora lutea were absent at the time of hysterectomy ovulation occurred and the newly formed corpora lutea persisted. Loeb suggested that the effect of the uterus upon the ovaries was indirect

and involved only the life of the corpora lutea.

In the rat, pituitary stalk-section during pseudopregnancy results in the maintenance of the corpora lutea and follicular regression; whereas luteal regression occurs in pituitary stalk-sectioned rabbits. These apparent species differences may be partially explained by the results of pituitary hormone injections following hypophysectomy. In the absence of the pituitary, prolactin maintains functional corpora lutea in the rat and luteinizing hormone maintains the corpora lutea in the rabbit. Neurohumoral substances produced in the hypothalamus stimulate gonadotropin (follicle stimulating hormone, luteinizing hormone) and inhibit prolactin (luteotropic hormone in the rat and mouse) production by the pituitary. Transection of the pituitary stalk in rats separates the pituitary from hypothalamic control and thus allows the production of prolactin.

In the sheep the corpora lutea regress following hypophysectomy and they are maintained for the duration of the estrous cycle after pituitary stalk-section. Hysterectomy results in the maintenance of the corpora lutea for a period of time equal to or exceeding the duration of pregnancy. The corpora lutea fail in hypophysectomized-hysterectomized sheep. Pituitary stalk-section combined with hysterectomy results in the maintenance of the corpora lutea for about 18 days. Thus, the sheep pituitary, separated from central nervous system control (hypothalamus), produces luteotropic hormone(s) capable of maintaining the corpora lutea. The uterus, however, produces a luteolytic effect which is dominant over the pituitary luteotropin.

Corpora lutea in the hypophysectomized pig are maintained for 12 to

14 days after estrus. Following hysterectomy they are maintained for a period of time equal to or exceeding the duration of pregnancy. The corpora lutea in hypophysectomized-hysterectomized pigs persist by injections of either luteinizing hormone or human chorionic gonadotropin. Luteinizing hormone or human chorionic gonadotropin does not maintain the corpora lutea beyond 14 days in hypophysectomized pigs which have the uterus intact.

The purpose of this investigation was to further elucidate the nature of factors involved in ovarian function and specifically the effect of pituitary stalk-section, during estrus, after mating or after hysterectomy, on the maintenance of the corpora lutea in the pig. The effect of exogenous gonadotropins on luteal function in hypophysectomized pigs is also presented.

LITERATURE REVIEW

Development and Regression of the Corpus Luteum

Three types of corpora lutea may be found following ovulation. Corpora lutea of the estrous cycle are formed from ruptured follicles at the time of ovulation. If fertilization of the ova or implantation of the blastocysts does not occur these corpora lutea soon degenerate. Corpora lutea of pseudopregnancy are formed, after nonfertile mating or mechanical stimulation of the cervix, in mammals such as the rat, mouse, rabbit and guinea pig. The third type of corpora lutea is that of pregnancy and parturition.

The rat and mouse show similar patterns in development and regression of their corpora lutea (Asdell, 1946). The estrous cycle lasts 4 or 5 days with nonfunctional corpora lutea being formed, developing for 2 to 3 days and then regressing. The corpora lutea of pseudopregnancy are physiologically active and produce progestins. Pseudopregnancy lasts 10 to 12 days in the mouse and 14 to 15 days in the rat. Regression of the corpora lutea commences about 3 days before the end of pseudopregnancy. During pregnancy the corpora lutea gradually increase in size for the first 10 to 14 days, remain until parturition and then begin a gradual regression.

Functional corpora lutea develop in the ovaries of the guinea pig following ovulation (Asdell, 1946). These corpora lutea increase in size for 10 to 11 days and then gradually regress. By 31 days after ovulation they are half of their original size. The corpora lutea of

pregnancy are functional for at least 26 days after ovulation and are still present at the time of parturition. Heap and Deanesly (1964) observed the continuation of pregnancy following ovariectomy as early as 20 days post coitum. In some guinea pigs abortion occurred 28 days post coitum.

Corpora lutea are rapidly formed after estrus in the golden hamster and regress after about 3 days. These corpora lutea of the cycle reach a maximum diameter of about 700μ (Asdell, 1946). If the hamster is pseudopregnant the corpora lutea attain a diameter of about 860μ and begin to regress at about day 7. Corpora lutea of pregnancy are maintained for the duration of pregnancy, reach a maximum size of 1000μ and regress rapidly after parturition.

The rabbit will breed at any time of the year but there are no estrous cycles, for sexual excitement or coitus is required for ovulation and the formation of corpora lutea (Asdell, 1946). The corpora lutea formed following ovulation begin to develop in about 6 hours and reach their full size within 8 days. Corpora lutea of pseudopregnancy last 16 to 17 days and luteal regression commences by the 18th day. They persist for the duration of pregnancy (30 to 32 days). These corpora lutea are gradually reabsorbed after parturition and somewhat more rapidly if the doe is lactating.

In the absence of a male the ferret remains in estrus for the duration of the breeding season. Ovulation and corpora lutea formation are induced only by coitus or cervical stimulation (Asdell, 1946). The

corpora lutea formed after ovulation persist for the duration of pregnancy or pseudopregnancy (42 days). The fully formed corpora lutea measure about 2 mm diameter with the ovary consisting almost entirely of luteal tissue. They degenerate rapidly at the end of pregnancy and there are no corpora lutea of lactation.

In the cat the corpora lutea develop only after coitus or cervical stimulation (Harrison, 1948). They are solid 7 days after ovulation and reach their maximum size at 10 to 15 days. The corpora lutea of pregnancy begin to regress at about day 20. Those of pseudopregnancy do not regress until about 28 days after ovulation although function ceases at 20 days. Corpora lutea of pregnancy become vacuolated at about day 50, persist throughout lactation and are found 6 to 8 months later.

The corpora lutea of the dog reach their maximum size at about day 16 and begin to regress at about day 30 after ovulation (Harrison, 1948). They are maintained for the duration of pregnancy (60 days).

Morphological changes in the ovary of the pig throughout the estrous cycle were reported by Corner (1921) and Burger (1952). Burger described the development and regression of the ovary in this species as follows:

During diestrus the ovarian weight increased from the 3rd until the 12th day after the onset of estrus. This was attributed primarily to the growth of the developing corpora lutea, which enlarged shortly after ovulation, reached a maximum diameter of about 11 mm by the 15th and regressed to about 4 mm by the 20th day. The corpora lutea gradually changed in color from a dark red on the 3rd day to a pale purple by the 15th day of the cycle. Between days 15 to 18 they were a yellowish cream

and with advancing age they became white. At the 3rd day the central cavities of the corpora lutea were filled with dark red blood clots, which were replaced with connective tissue plugs by the 6th day or by a yellowish fluid that persisted until the 15th to 18th days. A marked vascularity of the corpora lutea was evident from the 6th to 18th days. The corpora lutea of the preceding cycle did not change appreciably in diameter during diestrus, but they rapidly involuted at the onset of estrus and virtually disappeared when about 40 days old. The average diameter of the large follicles increased until the 18th day and reached a maximum diameter of about 9 mm. The total number of grossly visible follicles did not change during diestrus. Robinson and Nalbandov (1951), however, reported an increase in the number of follicles by the 6th to 8th days of the cycle. This was followed by a reduction and the maximum number of follicles was again observed by the 13th to 20th days.

The corpora lutea of early pregnancy in the pig remained about the same size as those of the 12th to 14th days of the cycle (Corner, 1915). Although histological changes were observed by Corner, the gross morphology remained constant until about the 110th day. At this time luteal regression began and was similar to that of the cycle.

Neill and Day (1964) reported that corpora lutea, induced during the luteal phase of the estrous cycle, persisted beyond the 15th day. These corpora lutea persisted 14 to 15 days from the time of formation. Thus the induced corpora lutea in the pig had a functional life of at least 14 to 15 days irrespective of the time of formation. The corpora

lutea formed at estrus began to regress at 14 to 15 days, whereas the induced corpora lutea remained functional. No differences were observed in the concentration of progesterone in the two sets of corpora lutea at the same stage of their respective development.

Progesterone production by the corpora lutea of the pig during the estrous cycle was investigated by Rombauts, Pupin and Terqui (1965) and Masuda, Anderson, Henricks, and Melampy (1966). Rombauts et al. (1965) observed an increase in progesterone concentration to 65 $\mu\text{g/g}$ by day 13 and a decrease to 4 $\mu\text{g/g}$ by day 15. The corpora lutea reached a maximum weight of 487 mg at day 13, followed by a decrease to 358 mg by day 15 and 120 mg at day 19. The progesterone concentration was 3.4 $\mu\text{g/g}$ at day 19. Masuda et al. (1966) reported a similar maximum progesterone concentration in the corpora lutea from days 8 to 12. This was followed by a decline to 41 $\mu\text{g/g}$ at day 14, and 15 $\mu\text{g/g}$ at days 16 and 18. Duncan, Bowerman, Hearn, and Melampy (1960) observed a similar progesterone concentration in the corpora lutea at day 12, a maximum progesterone concentration of 74 $\mu\text{g/g}$ at day 16 and no detectable level of progesterone by day 18.

Several authors reported that the progesterone concentration in the corpora lutea during pregnancy in pigs was similar to that found on day 14 of the estrous cycle (Loy, McShan, Self, and Casida, 1958; Spies, Zimmerman, Self, and Casida, 1960; Rigor, Self and Casida, 1963; Rombauts et al., 1965; Schultz, Speer, Hays, and Melampy, 1966). The concentration of progesterone as reported by the various authors ranged from 34 to 104 $\mu\text{g/g}$ of luteal tissue. Masuda et al. (1966) reported an increase

in luteal progesterone concentration from day 14 (65 $\mu\text{g/g}$) to day 18 (87 $\mu\text{g/g}$) and a decline on day 25 (63 $\mu\text{g/g}$) of pregnancy. Duncan et al. (1960) found an increase in luteal progesterone concentration from days 16 to 48 of pregnancy. Erb, Nofziger, Stromshak and Johnson (1962) reported a gradual decrease in luteal progesterone concentration during early pregnancy. A gradual decline in luteal progesterone concentration began 15 days before parturition (Duncan et al., 1960; Masuda et al., 1966).

Rigor et al. (1963) found that daily injections of estradiol-17 β from days 9 to 24 of pregnancy had no effect on luteal progesterone concentration although it did reduce the size of the corpora lutea. The injection of progesterone into pregnant pigs reduced the corpora lutea weight from 419 mg to 178 mg and reduced the progesterone concentration to 19 $\mu\text{g/g}$ (Spies et al., 1960). Single injections of progesterone into the corpora lutea had no effect on the progesterone content.

The corpus luteum of the doe and goat remains at a constant size for the duration of pregnancy, whereas in sheep it increases in size to about day 14. In these animals the corpus luteum is maintained until parturition (Asdell, 1946). Unlike the pig, experimentally induced corpora lutea in the sheep did not have a functional life independent of those already present (Inskeep, Oloufa, Pope, and Casida, 1963). Inskeep et al. (1963) observed that corpora lutea induced during the estrous cycle regressed at the same time as those formed at estrus and thus had no effect on the normal 16 to 18 day estrous cycle. This termination of luteal function in the ewe was also observed by Short (1964). Short attributed the cessation of luteal function to a dual control of the ovaries, pos-

tulating a uterine luteolysin which was dominant over a pituitary luteotropin by 14 days after estrus.

The corpus luteum of the cow increases in size until about days 16 to 18 of the estrous cycle and is followed by a decrease in size shortly before the animal returns to estrus (Hansel, 1959). The corpus luteum increases from 8 mm³ at 1 day after estrus to 20.5 mm³ at 18 days and decreases to 12.5 mm³ at the following estrus. In the event of pregnancy the corpus luteum persists without any change in size for the duration of pregnancy (Asdell, 1946; Catchpole, 1959). Bowerman and Melampy (1962) observed a gradual increase in corpus luteum weight from 4.3 g during the first 50 days to 7.4 g at 210 to 250 days of pregnancy and a decrease to 6.2 g at 250 to 280 days.

Measurement of the hormone content of the corpus luteum of the cow during pregnancy showed the presence of both progesterone and Δ^4 pregnen-20 β -ol-3-one (Gorski, Erb, Dickson, and Butler, 1958). Anderson, Bowerman and Melampy (1965a) reported a corpus luteum weight of 0.5 g and a progesterone concentration of 14 μ g/g 4 days after estrus. Small amounts (2.5 μ g/g) of Δ^4 pregnen-20 β -ol-3-one also were present. Bowerman and Melampy (1962) found that the mean corpus luteum progesterone concentration increased from 29.2 μ g/g at day 8 to 47.2 μ g/g at day 16 of the estrous cycle. Δ^4 pregnen-20 β -ol-3-one increased from 0.3 μ g/g at day 8 to 2.0 μ g/g by day 16. During pregnancy progesterone concentrations ranged from 4.0 to 18.2 μ g/g and Δ^4 pregnen-20 β -ol-3-one ranged from 3.3 to 10.5 μ g/g of luteal tissue.

The corpus luteum of the mare reaches its maximum size about 14 days after estrus and is physiologically active about 15 to 17 days (Harrison,

1948). The corpus luteum formed after mating begins to regress at about the 40th day of pregnancy. At this time ovarian follicles become luteinized or ovulate and form accessory corpora lutea. Regression of these corpora lutea begins on about the 150 day and only minute vestiges remain during the later stages of pregnancy.

The life of the corpus luteum in primates has been studied in relation to the menstrual cycle, with the time of menstruation coinciding with the cessation of luteal function (Asdell, 1946). In monkeys (Macaca mulatta) ovulation occurs at about day 13 of the cycle and a corpus luteum is formed from the ovulated follicle. The corpus luteum reaches a maximum diameter of about 5.5 mm and is functional for about 15 days. It is nonfunctional after menstruation. Abnormal degeneration, however, may follow and the structure may remain relatively large for an extended period of time. The normally degenerating corpus luteum is not visible after 4 menstrual cycles. The corpus luteum of pregnancy may persist for the duration of pregnancy but it appears not to be necessary after the 25th day.

Factors Affecting the Maintenance of the Corpus Luteum

The uterus and maintenance of the corpus luteum

Anderson, Bowerman and Melampy (1963b) in a review of the effects of hysterectomy during various reproductive stages found several species differences. Hysterectomy does not alter the estrous cycle in the mouse, rat, 13-lined ground squirrel, ferret, rabbit, opossum or dog. In the guinea pig, pig, sheep and cow there is a cessation of estrous cycles

and persistence of corpora lutea for an extended period of time equal at least to the duration of pregnancy. Hysterectomy performed during pseudopregnancy results in a prolongation of corpora lutea life in the golden hamster, rabbit and rat. In primates it appears that hysterectomy does not affect the life of the corpus luteum or the length of the menstrual cycle. Hysterectomy performed during pregnancy does not alter the life span of the corpora lutea of the rat or 13-lined ground squirrel, results in immediate luteal failure in the golden hamster, decreases corpora lutea life in the rabbit and increases luteal life in the pig. Furthermore, the prolongation of corpus luteum life is relative to the amount of uterine tissue remaining in partially hysterectomized guinea pigs, pigs and cows. Denervation of the uterus does not alter the life of the corpus luteum.

Silbiger and Rothchild (1963) reported that the duration of pseudopregnancy was longer in rats hysterectomized early in diestrus than when done late in diestrus. Pseudopregnancy was also increased by partial hysterectomy although not as long as with panhysterectomy.

Fisher (1965) reported that hemi-hysterectomy and ipsilateral ovariectomy did not alter the estrous cycle of the guinea pig (16 days), whereas hemi-hysterectomy and contralateral ovariectomy prolonged the estrous cycle to about 32 days. Lengthened estrous cycles occurred in hemi-hysterectomized animals. The ovaries showed a striking histological asymmetry which developed late in the lengthened cycles. Corpora lutea were maintained on the operated side, whereas they regressed as usual on the unoperated side. Bland and Donovan (1966) reported the

presence of smaller corpora lutea (1.97 mm^3) in the ovary adjacent to the intact uterine horn as compared to corpora lutea (3.10 mm^3) in the ovary on the side of hemi-hysterectomy in the guinea pig. The corpora lutea in the ovaries of sham operated females were similar to those in the ovary adjacent to the intact horn. Those in the ovaries of hysterectomized guinea pigs were similar to corpora lutea on the side of hemi-hysterectomy. The authors concluded that luteal function in the guinea pig was governed by a luteolytic factor produced by each uterine horn and acting on the neighboring ovary. Bland and Donovan (1965b) reported a similar effect with the placement of glass beads in one uterine horn. Bland and Donovan (1965a) observed normal estrous cycles following autotransplantation of part of the uterine horns. Transfer of blastocysts or conceptuses to the spleen at the appropriate stage of the cycle did not alter the estrous cycle. The transfer of blastocysts or conceptuses to autotransplanted uterine tissue delayed estrus up to 11 days. The authors postulated that normal placental development with the involvement of the endometrium was necessary for the abolition of estrus.

Heap and Deanesly (1964) determined blood progesterone levels in pregnant guinea pigs ovariectomized at the 28th day. Blood progesterone levels were lower at all times after ovariectomy as compared with the corresponding stage in intact animals, except at the end of pregnancy. The contribution of the placenta to the systemic blood progesterone level was found to be much less than that of the ovaries until near parturition. Progesterone production by the placenta, however, was sufficient to maintain pregnancy.

Removal of the fetus and placenta from intact rabbits resulted in re-

gression of the corpora lutea in a manner similar to the effect of hypophysectomy of the pregnant rabbit (Rennie, Davis and Friedrich, 1964). When the fetus was removed, but the placenta left intact, the corpora lutea remained functional and similar to those of pregnancy.

du Mesnil du Buisson and Dauzier (1959) reported persistence of corpora lutea for 117 days in hysterectomized pigs both pregnant and non-pregnant at the time of surgery. Ovulation occurred about 200 days after the estrus prior to hysterectomy. Anderson, Butcher and Melampy (1963a) reported maintenance of corpora lutea for at least 120 days when hysterectomy was performed during the active luteal phase of the estrous cycle. There was a return to estrus, ovulation and corpora lutea formation when hysterectomy was performed after the active luteal phase. The corpora lutea formed at this time persisted for at least 120 days. du Mesnil du Buisson (1961b) observed unilateral luteal regression when a small anterior portion of one uterine horn remained after partial hysterectomy. The corpora lutea in the contralateral ovary remained functional and the pigs did not return to estrus. Partial hysterectomy, where more than one quarter of one uterine horn and the cervix remained, resulted in estrous cycles of 4 to 10 days longer than normal (Anderson, Butcher and Melampy, 1961).

Anderson et al. (1961) reported that progesterone levels in corpora lutea decreased with time following hysterectomy of the pig. Progesterone concentration decreased from 80 $\mu\text{g/g}$ at 25 days to 30 $\mu\text{g/g}$ at 110 days after hysterectomy. Spies et al. (1960) reported that proges-

terone injections for 10 days reduced mean corpus luteum weight from 421 mg to 181 mg and luteal progesterone concentration from 42 $\mu\text{g/g}$ to 26 $\mu\text{g/g}$ 25 days after hysterectomy.

The quantity of nongravid uterine horn remaining in partially hysterectomized pigs had a pronounced effect on the maintenance of the corpora lutea (du Mesnil du Buisson, 1961a; Rathmacher, 1965; Anderson, Rathmacher and Melampy, 1966). When most of a nongravid uterine horn remained bilateral luteal regression occurred and pregnancy failed. Corpora lutea regression was unilateral and pregnancy was maintained in one horn when half or less of a nongravid uterine horn was present. Unilateral hysterectomy with the remaining horn gravid resulted in maintenance of functional corpora lutea on both ovaries. Pregnancy was maintained when the nongravid horn was removed before day 14, whereas pregnancy failed when it was removed after day 16. Exogenous progesterone maintained pregnancy for at least 25 days in pigs containing half or more of a nongravid uterine horn.

Removal of all of the fetuses and all except the anterior 20 cm of uterine horn, during mid-pregnancy, resulted in the maintenance of the corpora lutea similar to that of hysterectomy (du Mesnil du Buisson and Dautzier, 1959). du Mesnil du Buisson and Rombauts (1963b) reported the maintenance of pregnancy in subtotally hysterectomized pigs. One fetus and its corresponding section of uterine horn remained in situ. Removal of a number of embryos and the corresponding section of uterine horn, (at day 40) did not interrupt pregnancy. Pregnancy failed when the embryos, but not the corresponding section of uterine horn, were removed.

Moor and Rowson (1964) reported that a day 12 sheep embryo can be transferred to a nonpregnant day 12 recipient and maintain a normal pregnancy. If the transfer was made at day 13, pregnancy was not maintained and the recipient ewe showed a normal estrous cycle. The mated ewe with embryos removed at day 12 exhibited a normal estrous cycle, while flushing at day 13 or 14 resulted in a prolonged estrous cycle.

Hysterectomy of the heifer resulted in a cessation of estrous cycles (Wiltbank and Casida, 1956; Anderson, Neal and Melampy, 1962). Partial hysterectomy resulted in estrous cycles of normal length after one prolonged cycle. Anderson et al. (1965a) and Malven and Hansel (1964) observed a single estrus following enucleation of the corpora lutea of previously hysterectomized heifers. The progesterone concentration of the corpora lutea of hysterectomized heifers was similar to that of the mid-luteal phase of the estrous cycle ($32.5 \mu\text{g/g}$) while the Δ^4 pregnen-20 β -ol-3-one concentration doubled ($9.6 \mu\text{g/g}$).

The pituitary and maintenance of the corpus luteum

The effect of hypophysectomy on the ovaries of the rat was first observed by Smith (1926, 1930). Follicular atresia began almost immediately after hypophysectomy and no normal follicles were formed within 4 days. The life of the corpora lutea was abnormally prolonged and although regressing, still observed 9.5 months later. No estrous cycles or ovulations occurred during this period. Primordial follicles continued to develop but soon regressed. Selye (1933c) reported that hypophysectomy of the immature rat resulted in a rapid degeneration of

the theca cells, thus indicating that the pituitary had a direct effect on the ovaries before sexual maturity. Richter and Wislocki (1930) also observed an absence of estrous cycles in the hypophysectomized rat. When a small quantity of anterior pituitary tissue remained the cycles were irregular. Removal of the posterior pituitary had no effect on subsequent estrous cycles, pregnancy or parturition (Smith, 1932). During a 5 week period after hypophysectomy of the pseudopregnant rat Baird, Wolf and Rennels (1961) observed a continual decrease in ovarian weight while ovarian ascorbic acid concentration was maintained.

Daniel, Duchon and Prichard (1964) reported that the long term effects of pituitary stalk-section or hypophysectomy of the rat were essentially the same. The ovaries were reduced in weight from 70 mg to 14 mg at the time of sacrifice. No new corpora lutea were formed and there were no mature follicles. The corpora lutea were very small and showed extensive regression. Greep and Barrnett (1951) reported similar results 19 to 54 days after pituitary stalk-section. The corpora lutea were maintained although they were not functional. Functional corpora lutea were maintained for at least 74 days following pituitary stalk-section and the placement of a barrier to vascular regeneration (Nikitovitch-Winer, 1965). The follicular apparatus and interstitial tissues were atrophic. Rats having a defective barrier exhibited one pseudopregnancy followed by normal vaginal cycles. Pseudopregnancy was also observed merely by the manipulation of the pituitary stalk without transection.

Everett (1954) transplanted the pituitary to the kidney capsule or the fascia in the neck on the day of ovulation. The uterus was traumatized on the 4th day and the rats were killed on the 8th day. Follicular development was absent, and the follicular apparatus and interstitial tissue were atrophic. A strong decidual reaction was found in pituitary transplanted, but not in hypophysectomized rats. Large red corpora lutea similar to those of pseudopregnancy were found in the ovaries of pituitary transplanted rats. The corpora lutea were much smaller, pale and apparently regressing in the hypophysectomized rats. It was suggested that the transplanted pituitary, freed from hypothalamic control, secreted a luteotropic hormone. In later studies Everett (1956) found that the corpora lutea formed following ovulation and pituitary transplantation persisted for at least 3 to 4 months. If the transplanted pituitary were removed there was marked luteal regression within one week. Similar results were observed by Baird et al. (1961). Four weeks after hypophysectomy, however, there was a decrease in the ovarian ascorbic acid concentration.

Pituitary tissue, retransplanted from the kidney to the median eminence 3 to 4 weeks later, resulted in a return of normal estrous cycles in 8 to 68 days (Nikitovitch-Winer and Everett, 1958b). Estrous cycles were of normal duration and the rats became pregnant. None of these rats lactated although mammary development was observed. Transplantation of the pituitary to the temporal lobe of the brain produced no luteotropic effect.

Nikitovitch-Winer and Everett (1958a) reported that corpora lutea were maintained when the pituitary was transplanted at estrus, the 2nd

or 3rd day of diestrus, but not at proestrus. When the proestrous rats were induced to ovulate by gonadotropin injection the subsequently formed corpora lutea were maintained. The authors concluded that the corpora lutea at proestrus were regressing and unable to respond to the pituitary luteotropin. Uteri of the engrafted rats retained their reactivity to trauma for at least 42 days. The corpora lutea were maintained longer when the pituitary was transplanted to the kidney capsule than when it was transplanted to the eye.

Flament-Durand (1965) grafted pituitaries into the hypothalamus and subsequently hypophysectomized the rats. Eight of 93 rats became cyclic and possessed ovaries containing numerous follicles but no corpora lutea. The ovaries of the acyclic rats were similar to hypophysectomized control rats. Halász and Pupp (1965) severed all nervous connections to the hypophysotrophic area of the hypothalamus. One group of rats exhibited constant vaginal estrus with ovaries containing follicles and no corpora lutea, whereas the others showed only occasional estrous vaginal smears and ovaries containing persistent but not fresh corpora lutea.

The effect of hypophysectomy on the hypothalamus of the rat was most evident in the paraventricular nuclei (Frykman, 1942). There was a 35% reduction in the quantity of Nissl substance in the paraventricular nuclei and it was confined to a specific area of the nuclei.

Implantation was prevented in mated rats hypophysectomized not more than 4 days after coitus (Selye, Collip and Thomson, 1933b; Pencharz and Long, 1933). Death and reabsorption of the fetuses occurred if hypophy-

sectomy was performed at 7 to 10 days, while at 11 to 21 days pregnancy was maintained. The gestation period, however, was prolonged and the mothers died, or gave birth to dead or living young. At parturition lactation ceased within 24 hours. Collip, Selye and Thomson (1933) reported a brief period of lactation in hypophysectomized rats when the uterus was emptied during late pregnancy. Selye, Collip and Thomson (1933a) found milk secretion in immature rats following the injection of HCG and removal of the induced corpora lutea. Rats receiving the same treatment and also hypophysectomized did not lactate.

The effect of hypophysectomy on mice was reported by Bardin, Liebelt and Liebelt (1964) to be similar to that in rats. After 40 days the ovaries were atrophic and contained degenerating corpora lutea by histological examination. Hypophysectomized mice bearing hypophyseal isografts had corpora lutea indicative of a slower rate of regression. These mice showed a constant diestrous smear and were not responsive to uterine trauma. Intact mice bearing hypophyseal isografts showed a prolonged diestrous length. Castrated female mice with ovarian and pituitary tissue transplants to the eye exhibited vaginal cycles of pseudopregnancy length (Browning and White, 1962). Corpora lutea were formed and persisted for the duration of pseudopregnancy. For half of pseudopregnancy the corpora lutea were hyperemic, with the hyperemia disappearing two days before ovulation. Hoshino (1964) reported that transplantation of the pituitary gland from mature mice into immature females resulted in an earlier vaginal opening than found in unoperated controls. Animals with pituitaries from male mice had earlier vaginal opening than those with pituitaries

from female mice. The stage of the estrous cycle at which the pituitary was obtained for implantation had no effect on vaginal opening.

Newton and Beck (1939) and Gardner and Allen (1942) reported that hypophysectomy of mice did not affect the maintenance of pregnancy. The ovaries appeared normal at the end of gestation and the young born were normal. Milk production was sufficient to keep the young alive for only 36 hours.

Hypophysectomy of immature guinea pigs resulted in a rapid regression of the ovaries to half of their original size in 4 to 6 days (Perry and Rowlands, 1963a). The follicles decreased in number and generally atrophied. Dempsey (1937) hypophysectomized guinea pigs immediately after ovulation. Healthy corpora lutea, small normal follicles and many atretic vesicular follicles were found 12 days later. Rowlands (1962) reported that hypophysectomy of previously hysterectomized guinea pigs did not result in regression of the corpora lutea for at least 21 days. Only atretic Graafian follicles were found after hypophysectomy. Perry and Rowlands (1963b) and Heap, Perry and Rowlands (1965) reported that hypophysectomy 2 to 5 days after estrus had only a limited effect on the corpora lutea. These corpora lutea grew for a longer period of time than those of the estrous cycle, reached a maximum size of 1.7 mm^3 by day 30. In contrast, hypophysectomy at day 10, when the corpora lutea had reached their maximum size of 2 mm^3 , resulted in luteal regression almost as rapid as that found during the estrous cycle. The progesterone content of corpora lutea of guinea pigs hypophysectomized shortly after estrus

and removed 1 to 8 days later was higher than that found during the luteal phase of the estrous cycle or pregnancy, while the plasma progesterone level was lower.

Haterius (1937) hypophysectomized guinea pigs and transplanted the pituitary to the anterior chamber of the eye. Unlike the rat, the ovaries became progressively more follicular and after about 2 months a condition of constant estrus supervened. The constant estrus was characterized by a continual estrous vaginal smear, markedly hypertrophied uteri and well proliferated mammary glands. The effect upon the ovaries was exclusively follicle stimulating, but there were no fully matured follicles and no ovulations. The author suggested that the follicles developed, subsequently regressed and were followed by new growth of follicles.

Implantation of blastocysts in the uterus of guinea pigs hypophysectomized 2 days after mating was reported by Perry and Rowlands (1963b). In some cases the corpora lutea grew to the size of those in normal pregnancy. Pencharz and Lyons (1934) observed that fetal reabsorption began within 2 days in guinea pigs hypophysectomized on day 34 to 36. Normal gestation occurred when hypophysectomy was performed at the 40th or 41st day. Only a slight and transient milk secretion occurred at parturition. The corpora lutea regressed rapidly after parturition.

Smith and White (1931) reported that hypophysectomy shortly after coitus did not affect the development of the corpora lutea of the rabbit for the next 2 days. The corpora lutea remained the same at 4 days and began to regress within 8 days. White (1933a) found that the large

ovarian follicles decreased in size 3 days after hypophysectomy. Only small follicles were visible 6 days after hypophysectomy and they were detected microscopically several weeks later. The interstitial tissue did not appear to change while the ovaries were reduced to one third of their normal weight. The corpora lutea of rabbits hypophysectomized 7 days after ovulation showed rapid regression to almost indistinguishable grey elevations on the surface of the ovary 7 days later (Kilpatrick, Armstrong and Greep, 1964). Fee and Parkes (1929) and Firor (1933) found that ovulation, which normally takes place about 10 hours after copulation, was prevented by hypophysectomizing rabbits within 1 hour after coitus. If the operation was performed longer than 1 hour after coitus, ovulation occurred and the ovaries underwent normal but slower development. When ovulation did not occur follicular regression began about the time they should have ovulated. Deanesly, Fee and Parkes (1930) observed that the corpora lutea of rabbits hypophysectomized immediately after coitus were still functional 36 hours later.

Sectioning the pituitary stalk of rabbits or damaging it by lesions was found by Donovan and Harris (1955) to result in a suppression of estrus and general gonadal atrophy. White (1932) reported that blocking of pituitary blood circulation with wax caused infarction of the pituitary and prevented pregnancy.

Smith and White (1931), White (1933a) and Firor (1933) reported a failure of implantation or of pregnancy in rabbits hypophysectomized 3 to 26 days after coitus. The corpora lutea appeared to be regressing 4

to 10 days after hypophysectomy. Hypophysectomy at mid-pregnancy resulted in reabsorption or abortion of the fetuses. In later pregnancy abortion occurred within 2 days and the young died within 1 hour. The corpora lutea of pregnancy were difficult to identify 3 to 5 weeks after hypophysectomy. Histologically normal mammary glands persisted after sectioning of the pituitary stalk of lactating rabbits (Jacobsohn, 1949). Milk production or let down, however, was inhibited for the suckling young died within 2 days.

Hill and Parkes (1932) and McPhail (1933a) reported that ovulation was prevented by hypophysectomizing ferrets within 110 minutes of the beginning of mating. Ovulation occurred and corpora lutea were formed after this time, but the corpora lutea did not increase in size and appeared nonfunctional as indicated by a lack of uterine changes characteristic of pseudopregnancy. Implantation of blastocysts did not occur. According to Donovan and Harris (1954, 1955, 1956) general gonadal atrophy occurred following sectioning or destruction of the pituitary stalk of ferrets. Holmes (1961) reported a 95% reduction in the volume of the pituitary following stalk-section. Corpora lutea were maintained for at least 6 weeks after pituitary stalk-section and the placement of a barrier to neuro-vascular regeneration (Donovan, 1963a).

Donovan (1963b) reported a decrease in uterine size, ovarian weight and luteal weight after hypophysectomy of the pseudopregnant ferret. The corpora lutea ceased to function and soon regressed. Pituitary stalk-section of pseudopregnant ferrets resulted in regression of the follicles. Corpora lutea were maintained for about 4 weeks and then regressed. The

luteal regression was not observed in intact pseudopregnant ferrets. The author suggested that a pituitary luteotropin, along with an unidentified factor, was required for optimal luteal activity during pseudopregnancy.

McPhail (1935a) found that fetal absorption occurred after hypophysectomizing ferrets at the 21st day of pregnancy. The birth of dead or living young occurred 3 to 8 days after hypophysectomy at day 35. Luteal regression appeared normal and similar to that observed following pseudopregnancy. Hypophysectomy during lactation terminated milk production.

McPhail (1935b) reported that cats hypophysectomized at mid-gestation aborted, whereas they give birth to living young when the operation was performed in late gestation. Allen and Wiles (1932) also found that pregnancy was maintained in hypophysectomized cats. Lactation was absent, however, and the hypophysectomized mothers lacked the desire to suckle the young.

Initial observations on the effects of hypophysectomy on the pig were made by Robinson (1937) and St. Clair (1945). Both authors reported a complete lack of gonadal development or function in pigs sacrificed a few weeks to 12 months after hypophysectomy. du Mesnil du Buisson and Léglise (1963a) reported that hypophysectomy of the pig shortly after the beginning of estrus or during the luteal phase did not affect the duration of life of the corpora lutea of that cycle. The corpora lutea were smaller than found at the corresponding day in unoperated animals and follicular development was absent. Luteal tissue progesterone concentrations were normal 3 or 8 days following hypophysectomy (8 or 14 days after estrus). Twenty-two days after estrus the corpora lutea had re-

gressed in these animals as in intact cycling pigs. Pigs hysterectomized during the luteal phase of the estrous cycle and hypophysectomized 15 to 20 days later showed rapid luteal regression. The corpora lutea regressed from the 400 to 500 mg weight of the hysterectomized pig to 172 mg by 5 days and 50 to 80 mg by 10 or 11 days following hypophysectomy. du Mesnil du Buisson, Léglise, Anderson, and Rombauts (1964a) hypophysectomized pigs 4 days after mating. Six of 8 animals were pregnant 11 and 12 days following mating. The ovaries contained normal corpora lutea with luteal progesterone concentrations of 41 $\mu\text{g/g}$.

Denamur and Mauléon (1963a,b) reported that hypophysectomy of supra-ovulated prepubertal lambs did not affect the function of the corpora lutea. Histological examination indicated that the corpora lutea remained functional for 12 days and were similar to control animals. Kaltenbach, Niswender, Graber, and Nalbandov (1966) found smaller corpora lutea in ewes hypophysectomized on the day after estrus and ovariectomized at day 8 as compared with control animals. There was no difference in the luteal progesterone concentration of these two groups. Ewes hypophysectomized at day 5 and ovariectomized at day 12 had smaller corpora lutea than either day 5 or day 12 control animals. The progesterone content of the corpora lutea of the hypophysectomized ewes was also lower. Denamur and Mauléon (1963a) reported that luteal regression 16 days after estrus was more advanced in the hypophysectomized than in the control animals and essentially complete at 20 days. Denamur, Martinet and Short (1966) reported that hypophysectomy performed during the luteal phase of the estrous cycle resulted in a cessation of luteal function by day 12. A similar

result occurred when the ewe was hysterectomized at the same time. Animals hysterectomized during the luteal phase and hypophysectomized 20 to 30 days later showed a rapid decline in luteal function as evident by reduced progesterone secretion 2 days after hypophysectomy. Denamur and Mauléon (1963a) reported that the rate of luteal regression was slower in hysterectomized-hypophysectomized sheep than in hypophysectomized animals with the uterus intact.

Pituitary stalk-section of the sheep performed during the luteal phase of the estrous cycle resulted in the maintenance of the corpus luteum to day 15 (Denamur et al., 1966). This corpus luteum was similar to that found in intact ewes at day 15 of the estrous cycle. Pituitary stalk-section and hysterectomy performed at the same time during the luteal phase of the cycle resulted in the prolongation of luteal function to day 18. When the pituitary was stalk-sectioned 20 to 30 days after hysterectomy the corpora lutea continued to secrete progesterone for at least 15 days. The authors suggested that the pituitary secreted a luteotropic hormone, at least for a period of time, after pituitary stalk-section. In addition, the corpus luteum was under the dual control of the pituitary and the uterus, and a uterine luteolysin was dominant to the pituitary luteotropic by day 15 of the cycle.

Hypophysectomy, ovariectomy followed by hypophysectomy or pituitary stalk-section did not interfere with the course of pregnancy in sheep (Denamur and Martinet, 1961; Adams, Daniel and Prichard, 1963). Cowie, Daniel, Prichard and Tindal (1963) reported that either hypophysectomy or pituitary stalk-section of goats during pregnancy resulted in abortion

within a few days.

Complete cessation of milk production 2 days after hypophysectomy of the goat was reported by Hill, Turner, Urer and Gomez (1935). Cowie and Tindal (1961) found that milk production was one-fifth or less within 10 days of hypophysectomy.

Daniel and Prichard (1957) found extensive necrosis of the anterior pituitary after sectioning of the pituitary stalk. The necrosis was remarkably uniform indicating only one major blood supply to the sheep pituitary. Cowie et al. (1963) suggested that the viable tissue was insufficient and unable to produce the gonadotropic hormones required to sustain normal reproductive function.

The hormonal control of the corpus luteum

Smith (1930) reported that daily injection of a saline extract of rat anterior pituitary tissue into hypophysectomized rats resulted in a return to an estrous condition within 5 days. In the ovaries atresia ceased, follicles developed and luteal cysts occurred. A cessation of the injections resulted in a rapid regression of the ovaries, similar to the effect of hypophysectomy. Van Dyke (1936) in his review reported a 10-fold increase in ovarian weight of intact rats receiving injections of anterior pituitary extracts. The increase in ovarian weight was attributed to hyperemia associated with many large cystic follicles. These cysts degenerated and formed corpora lutea larger than found in the normal rat.

Williams (1945a,b,c,d), and Rowlands and Williams (1946) found that

serum gonadotropin injection induced ovulation in immature rats hypophysectomized 1 to 4 days previously. Ovaries increased in weight until day 7 after hypophysectomy and after that they became refractory to serum gonadotropin injections. Ovulation was produced in hypophysectomized mature rats by injection of serum gonadotropin from days 6 to 9 and followed 4 days later by a single injection of urinary gonadotropin. Diethylstilbestrol caused partial maintenance of the ovaries of hypophysectomized immature rats. Along with the decreased rate of gonadal atrophy the ovaries responded to serum gonadotropin 10 days after hypophysectomy. Tyndale and Levin (1937) found that menopause urine injections increased ovarian weight in hypophysectomized rats more than in unoperated controls. There was marked follicular growth with little or no luteinization. Desclin (1949) observed that diethylstilbestrol had no effect on the maintenance of the corpora lutea of rats hypophysectomized at the start of injection. The corpora lutea regressed in a manner similar to that of hypophysectomized control rats. Diethylstilbestrol, however, did maintain large follicles on the ovaries. Gaarenstroom and de Jongh (1946) reported luteal failure in hypophysectomized rats receiving estrogen injections. The injection of estrogen plus pituitary extract maintained corpora lutea and progesterone production. Moricard (1953) found follicular growth with cavity formation 144 hours after the first injection of pregnant mare serum gonadotropin (PMSG) into hypophysectomized immature rats. The ovaries of these rats were capable of forming corpora lutea 30 days after hypophysectomy. Injection of human chorionic gonadotropin (HCG) resulted in increased ovarian size due only to increased interstitial tissue. The

formation of corpora lutea did not occur following follicle stimulating hormone (FSH) injection of hypophysectomized rats (Simpson, Li and Evans, 1951). The injection of FSH plus a small quantity of HCG resulted in the formation of corpora lutea. Carter, Woods and Simpson (1961) used highly purified FSH and interstitial cell stimulating hormone (ICSH or luteinizing hormone, LH) to develop follicular growth and multiple ovulation respectively in hypophysectomized rats.

Smith and Bradbury (1966) reported that the ovaries of hypophysectomized immature rats receiving human menopausal gonadotropin (HMG) injections after pretreatment with diethylstilbestrol or diethylstilbestrol and progestins had larger follicles than with progestin or no hormone pretreatment. No corpora lutea were formed after the HMG treatment alone. The injection of HMG and LH resulted in the formation of corpora lutea.

Astwood (1941) fractionated sheep pituitary glands and effectively separated follicle stimulating, thyrotropic, adrenotropic and lactogenic hormones. These preparations along with an acetone dried sheep pituitary suspension were studied for luteotropic activity in immature pseudopregnant hypophysectomized rats. Daily injections of estradiol were given after hypophysectomy to maintain a vaginal mucification when the corpora lutea were active. Estrogen did not affect the maintenance of the corpora lutea. Untreated rats showed permanent vaginal cornification. Crude pituitary extracts maintained a diestrous condition by development of successive sets of corpora lutea. A similar effect was found with the injection of rat placental extract. Pituitary residues with FSH and LH removed, maintained the corpora lutea while the thyroid gland and repro-

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ductive organs decreased in weight. Adrenotropic hormone was also removed without changing luteotropic activity. Some of the fractions which maintained the corpora lutea also induced mammary development. Thus, a luteotropin was separated from FSH, LH and thyrotropin, distinct from growth and adrenotropic factors, and not responsible for proliferation of mammary lobules in hypophysectomized rats.

The observations of Astwood were confirmed by Evans, Simpson, and Lyons (1941a) and Evans, Simpson, Lyons, and Turpeinen (1941b) using similar hormone preparations and studying placentoma formation in the traumatized uterus. Crude extracts of rat pituitary tissue produced excessive follicular growth in the ovaries. Extracts of ovine pituitary tissue produced follicular growth and corpora lutea formation. There was no placentoma formation in the uterus of these rats. Similarly FSH, ICSH, PMSG or HCG did not produce placentoma and maintenance of the corpora lutea in normal, hypophysectomized or adrenalectomized-hypophysectomized rats. No placentoma formation was found in gonadectomized rats. Lactogenic hormone was without effect on the corpora lutea if injections were started 11 days after hypophysectomy, unless corpora lutea were induced at that time by means of gonadotropic hormone injection.

Everett (1944) reported that prolactin stimulated progesterone production following induced corpora lutea formation in constant estrous rats. Prolactin maintained luteal progesterone production in hypophysectomized rats (Nelson and Cutuly, 1942; Tobin, 1942).

Transplantation of a portion of the pituitary gland to the kidney capsule resulted in estrous cycles of pseudopregnancy length (Alloiteau,

1958b). This, the author stated, was indicative of luteotropin release by the pituitary tissue in the kidney capsule in addition to the release of gonadotropins, for ovulation, from the pituitary tissue remaining adjacent to the hypothalamus. Alloiteau and Vignal (1958c) found that prolactin injections during the 4 days following estrus induced pseudo-pregnancy. This was attributed to prolactin stimulation of progesterone production and secretion, which in turn favored more prolactin secretion. Alloiteau and Acker (1960) reported that androgens had no effect on progesterone production by the corpora lutea of the rat.

Hypophysectomized immature rats given daily prolactin injections or with an autografted pituitary gland showed a deciduoma reaction indicative of normal corpora lutea function (Rennels and Guillet, 1963). Desclin and Koulischer (1960) found that pituitary tissue transplanted to the kidney capsule contained more prolactin when the rats received daily injections of estradiol benzoate. Estrogen implants in intact or pituitary transplanted rats caused hypertrophy of the corpora lutea (Desclin, 1950, 1956; Everett, 1956). The corpora lutea were maintained for 3 months. Desclin (1950) reported that estrogen implants did not prevent luteal regression in hypophysectomized rats. Rothchild and Schwartz (1965b) found a reduced rate of luteal regression in intact rats receiving estradiol. Estradiol, progesterone or estradiol and progesterone did not maintain corpora lutea in hypophysectomized rats. Corpora lutea were maintained in estrogen and progesterone treated intact rats and in hypophysectomized rats bearing pituitary transplants.

Rothchild (1965a) reported that LH administered to hypophysectomized

rats bearing autotransplanted pituitaries induced luteolysis. The degree of effect was related to the quantity of LH injected. Regression of the corpora lutea did not occur following injection of FSH, HCG, adrenocorticotrophic hormone (ACTH), or ovine prolactin. Administration of FSH increased the size of the corpora lutea. Rothchild and Schwartz (1965b) reported that LH induced regression of the corpora lutea of intact rats receiving estrogen and progesterone.

Baird et al. (1961) found that LH had no effect on the concentration of ascorbic acid in pseudopregnant rat ovaries for 5 weeks after hypophysectomy. When the pituitary was transplanted after hypophysectomy LH injection resulted in an even greater depletion of ovarian ascorbic acid than found in intact pseudopregnant rats.

Nikitovitch-Winer and Everett (1958b) reported that rats showed gonadotropin secretion when the pituitary was retransplanted to the median eminence 3 or 4 weeks after the initial autotransplantation and then primed with FSH and LH. Seven of 10 of these animals resumed estrous cycles while the others remained in constant estrus. None of the rats maintained pregnancy.

Nikitovitch-Winer and Everett (1965) observed that sectioning of the pituitary stalk and placement of a barrier to vascular regeneration prevented the secretion of FSH and LH while luteotropic hormone (LTH) secretion continued.

Wolthuis (1963) studied corpus luteum cell size in the PMSG, HCG primed hypophysectomized immature rat. Rats possessing a pituitary transplant or receiving a daily injection of pituitary homogenate had larger

luteal cells than hypophysectomized controls receiving PMSG and HCG. Purified prolactin plus PMSG and HCG gave similar results. The author suggested that under these conditions prolactin specifically stimulated an increase in the size of the rat corpora lutea.

Schlough, Schuetz and Meyer (1965) reported that the implantation of embryos in the progesterone-treated hypophysectomized rat occurred following injection of pituitary homogenates, HCG or PMSG. Implantation did not occur when purified LH, FSH, or LH and FSH were injected except at very high levels, or when the rats were ovariectomized prior to gonadotropin treatment. The quantity of pituitary homogenate, HCG or PMSG required for implantation was one tenth of the level of LH or FSH activity required for implantation.

Cutuly (1942) reported the maintenance of pregnancy in hypophysectomized rats with the injection of pituitary gonadotropin plus Lh. The corpora lutea and progesterone production were maintained by this hormone treatment. Serum gonadotropin alone did not maintain a normal pregnancy as shown by fetal reabsorption and prolonged gestations of 28 days. Averill, Ray and Lyons (1950) found that subcutaneous injections of day 12 rat placenta maintained pregnancy in rats hypophysectomized on the 6th day after mating. Injections were given for 6 days and consisted of 5 placenta, 275 mg placental tissue or 1 placenta and 0.5 mg estrone per day. Pregnancy was not maintained if the rats were also ovariectomized. Sammelwitz, Aldred and Nalbandov (1961) reported that progesterone injections, commencing at the time of ovulation, had no effect on the corpora lutea of pregnancy. Alloiteau (1957, 1958a) found that progesterone in-

jections beginning on day 6, the day of hypophysectomy, maintained pregnancy but not functional corpora lutea. Exogenous prolactin maintained the corpora lutea and pregnancy. Alloiteau and Bouhours (1964, 1965a,b) reported that HCG, PMSG, HCG and PMSG, or LH injected from days 8 to 12 maintained pregnancy in rats hypophysectomized on day 8. FSH or testosterone was without effect in maintaining the corpora lutea. The authors postulated a physiological role for LH from the 9th to 12th days of pregnancy by its sustaining secretion of estrogen which, along with a placental factor, maintained progesterone production by the corpora lutea. Estrogen injections from days 8 to 12 maintained pregnancy to day 13, at which time the authors suggested that placental hormones were sufficiently developed to stimulate progesterone production by the corpora lutea. MacDonald and Greep (1966) reported that exogenous LH stimulated estrogen secretion from the ovaries of hypophysectomized or hypophysectomized-autografted mated rats. The estrogen induced blastocyst implantation. Injection of progesterone or prolactin maintained viable blastocysts without implantation. Implantation occurred when LH was administered along with progesterone or prolactin. The authors suggested that the estrogen required for implantation was secreted in response to pituitary LH and that the autotransplanted pituitary did not contribute sufficient LH to induce implantation.

Injection of anterior pituitary extracts resulted in a 19-fold increase in the size of the ovaries of mice (Van Dyke, 1936). There was initially an ovarian hyperemia followed by follicular growth, maturation, the liberation of large numbers of ova and the formation of corpora lutea

of normal size. Dresel (1935) reported that prolactin injections commencing at estrus inhibited the estrous cycle of mice for 20 to 25 days. This was followed by 4 to 8 days of estrus even though prolactin injections continued.

Selye, Collip and Thomson (1933d) reported that hypophysectomized mice given injections of pregnancy urine developed theca cell luteinization without formation of normal corpora lutea. Kovačič (1964) found corpora lutea maintenance with prolactin injection of hypophysectomized mice. In further studies the ovaries of prolactin treated hypophysectomized mice showed no follicular growth or interstitial cell development. Graafian follicles developed while the interstitial cells and corpora lutea regressed during FSH injection of hypophysectomized mice. The injection of LH resulted in the return of the interstitial tissue, to a normal prehypophysectomy condition. Luteal maintenance and follicular growth were found at high levels of LH. Growth hormone was without effect on the ovaries but did produce a uterine deciduomata. Thyrotropin and ACTH were without effect on the uterus or ovaries. A uterine decidual response, some follicle development and partial luteal maintenance were produced with high levels of HCG. The injection of PMSG produced a decidual response in some instances. Follicular growth and interstitial tissue development were normal, and numerous corpora lutea were present at high levels of PMSG injection.

Van Dyke (1936) reported ovulation and corpora lutea formation in the guinea pig with rat, rabbit or homoplastic pituitary implants. Pituitary implants or pituitary extracts from other animals appeared to cause

follicular atresia, luteinization of the theca interna and growth of interstitial tissue. Large quantities of ovine and bovine pituitary extracts, contrary to the above observations, produced ovulation and formation of corpora lutea in estrous, immature, pseudopregnant and pregnant guinea pigs (Van Dyke, 1937). Rowlands (1962) found that the corpora lutea of hysterectomized guinea pigs were not affected by the injection of estradiol benzoate. Perry and Rowlands (1963a) reported that hypophysectomized immature guinea pigs were refractory to stimulation by gonadotropic hormones.

Stromshak and Casida (1964a,b) found that injection of 500 μ g of FSH had no effect on the corpora lutea of pseudopregnant rabbits. However, injection of 2000 μ g of FSH produced ovulation and regression of the original corpora lutea. Injection of 200 IU of HCG or 15 to 200 μ g of LH produced the same result.

Firor (1933) reported that pregnancy urine injection induced ovulation in rabbits, hypophysectomized 35 minutes after coitus. White and Leonard (1933b) induced ovulation with the injection of HCG or anterior lobe extract immediately after hypophysectomy. The corpora lutea formed were functional but smaller than found in normal rabbits. Follicular stimulation and cystic follicles were produced by injecting sheep anterior pituitary extract within 29 days after hypophysectomy. Human chorionic gonadotropin gave no response except at very high dose levels.

Robson (1937a) reported a failure of ovulation in estrous rabbits given a single injection of pregnancy urine, horse pituitary powder or

rabbit pituitary powder 3 days after hypophysectomy. One week after hypophysectomy the ovaries were refractory to gonadotropin injection. Daily injections of small quantities of pregnancy urine resulted in follicular maintenance for at least 8 days. Ovulation and corpora lutea formation were induced at this time with the injection of 2.5 to 5 mg of pregnancy urine. Daily injection of 10 mg of pregnancy urine in the hypophysectomized estrous rabbit resulted in the formation and maintenance of corpora lutea for 5 days. Similarly pregnancy urine injections maintained corpora lutea for 8 days in hypophysectomized pseudopregnant rabbits. Robson (1937c) reported maintenance of corpora lutea in pseudopregnant-hypophysectomized rabbits with the daily injection of 10 μ g of estrone.

In contrast to the effect of lactogenic hormone (Prolactin) in mice and rats Kilpatrick, Armstrong and Greep (1962) found it to be without effect in maintaining corpora lutea in the rabbit. Prolactin induced mammary growth and milk production. The LH injection of rabbits, commencing 1 week after ovulation, maintained the corpora lutea and a progestational endometrium, while the mammary glands were atrophied. In further studies Kilpatrick et al. (1964) found a second or new set of corpora lutea when LH injection was started 2 hours after hypophysectomy. The rabbits were killed 1 week after hypophysectomy and showed 2 distinct groups of corpora lutea. One group of corpora lutea was red and clearly younger, and the second group greyish white and obviously formed prior to hypophysectomy. Rabbits which received their first LH injection 12 hours after hypophysectomy had no new corpora lutea 7 days later. These

corpora lutea were similar to those of rabbits killed at day 14 of pseudopregnancy. Rennie et al. (1964) also reported that prolactin failed to maintain corpora lutea in the hypophysectomized rabbit. The rabbits were hypophysectomized at various stages of pregnancy or pseudopregnancy, given prolactin injections for 6 to 7 days and then the ovaries were examined.

Progesterone given daily, according to Robson (1937b), maintained pregnancy in rabbits hypophysectomized 3 days after ovulation. The corpora lutea regressed. Injections of pregnancy urine gonadotropin maintained both pregnancy and corpora lutea in rabbits hypophysectomized at various stages of pregnancy. Cessation of the gonadotropin injections resulted in luteal failure and fetal death within 48 hours.

Anterior pituitary extract injection of ferrets hypophysectomized 5 weeks previously was reported by McPhail (1933b) to have a limited effect on the ovaries. Follicular growth and theca luteinization occurred, but none of the follicles approached ovulatory size. McPhail (1935a) found that anterior pituitary extract injection of ferrets hypophysectomized in late pregnancy resulted in hypertrophy of the mammary glands and milk secretion, both before and after delivery of young.

The effects of various hormones on the maintenance of corpora lutea in hypophysectomized pigs were reported by Anderson, Léglise, du Mesnil du Buisson and Rombauts (1965b). Pigs were hypophysectomized 3 to 10 days following estrus and the ovaries were removed 20 days after estrus. Ovine pituitary powder (250 mg/day), HCG (1000 IU/day) or LH (bovine or equine; 5 mg/day) injected from days 12 to 20 did not maintain normal corpora lutea in the ovaries. The corpora lutea were regressing as shown by a

pale yellow color and an average progesterone concentration of 10 $\mu\text{g/g}$ of tissue but were larger than those found at day 20 in intact animals. Pigs receiving the above treatments and also hysterectomized maintained functional corpora lutea with progesterone concentrations of 34 to 84 $\mu\text{g/g}$ of tissue. Ovaries removed 27 to 33 days after estrus showed regressing corpora lutea except for the pigs receiving bovine LH (NIH-LH). Prolactin of bovine or porcine origin was without effect in maintaining the corpora lutea of hypophysectomized pigs. The authors stated that there was an interaction between the uterus and the pituitary whereby the uterus prevented utilization of the luteal stimulating hormone by the corpora lutea.

Gardner, First and Casida (1963) reported that corpora lutea were maintained in the pig with injection of estradiol-17 β or estrone from days 11 to 33 following estrus. The corpora lutea were smaller than those of pigs 11 days post estrus but contained a higher concentration of progesterone such that total progesterone content was the same.

Brinkley, Norton and Nalbandov (1964) reported that hypothalamic blocking with progesterone injections, beginning before or during estrus, did not prevent the formation of corpora lutea following ovulation. When progesterone injections were started before estrus they did prevent ovulation in some cases. The progesterone concentration was lower when injections of progesterone were started before, on the day of or the day after observed estrus. Sammelwitz et al. (1961) found normal corpora lutea in pigs 10 to 13 days pregnant and receiving progesterone injections beginning on the day of ovulation. The corpora lutea regressed if progesterone injections were continued beyond days 12 to 16.

du Mesnil du Buisson et al. (1964a) reported the maintenance of pregnancy in hypophysectomized pigs with the daily injection of 400 mg progesterone plus 0.15 mg estradiol-17 β from days 12 to 32 and 300 mg of progesterone from days 33 to 125. Two fetuses 25 and 27 cm long, 587 and 461 g were found on day 125. The corpora lutea in the ovaries had regressed.

The use of exogenous hormones to maintain the corpus luteum in the hypophysectomized sheep was reported by Denamur and Mauléon (1963a,b). Estrogen injection did not maintain the corpus luteum in the hypophysectomized animal although it maintained the corpus luteum in the intact ewe for about 50 days.

Cowie and Tindal (1961) studied the hormonal control of lactation in the goat following hypophysectomy. Daily injection of anterior pituitary extracts, growth hormone, prolactin, ACTH, corticoids, insulin or triiodo-L-thyronine all increased milk production, after a post hypophysectomy decline in milk production but the goats did not reach their pre-operative production level. Oxytocin was given just prior to milking to insure milk ejection.

Anterior pituitary extracts injected into the monkey (Macaca mulatta) resulted in follicular growth and formation of luteal tissue without ovulation (Van Dyke, 1936). Knobil, Kostyo and Greep (1959) reported excessive follicular growth and tissue luteinization by the pituitary tissue injections, beginning 2 to 12 months after the monkeys were hypophysectomized. The same result was observed when 5 to 10 IU per day of FSH and 500 to 2500 IU per day of HCG were substituted for the pitu-

itary homogenate. Multiple ovulation occurred when 1 IU of FSH was injected twice daily for 6 days and followed by twice daily injection of 0.1 IU of FSH plus 500 IU of HCG. Thus, the monkey ovaries were able to respond to gonadotropins one year after hypophysectomy. van Wagenen and Simpson (1957) and Simpson and van Wagenen (1958) produced multiple ovulation and subsequent corpora lutea formation in hypophysectomized monkeys with the injection of FSH followed by ICSH or HCG.

Gemzell (1961) observed induction of ovulation in women with the injection of FSH and HCG. A large number of follicular cysts and luteinized follicles were usually formed. Corpora lutea also were formed following ovulation. When greater numbers of follicular cysts were formed the author assumed that the woman's pituitary was more functional. Gemzell and Kjessler (1964) observed that the ovaries of the hypophysectomized woman were capable of normal function 3 years after hypophysectomy. Within 10 days of the beginning of injection of FSH and LH the ovary delivered a normal ovum which could result in pregnancy. If the woman became pregnant the corpus luteum so formed was maintained for the duration of pregnancy without exogenous gonadotropin therapy.

EXPERIMENTAL PROCEDURE

Crossbred pigs 6 to 8 months of age weighing an average of 100 kg (range 80 to 114 kg) were used as experimental animals. They were checked daily for estrus with vasectomized boars. The first day of estrus was designated day 1 of the estrous cycle. The animals were fed 2.3 kg of a 14 percent protein ration per day.

The pig was isolated and fasted for 18 to 24 hours before surgery. Immediately prior to the operation the animal was given an IV (intravenous) injection of atropine sulfate (0.4 mg; Eli Lilly and Co.), and an IM (intramuscular) injection of cortisone acetate (50 mg; Merck and Co.) and an IM injection of hydrochlorothiazide (100 mg; Merck and Co.). Thiopental sodium (1 to 2 g; Abbott Laboratories) was injected IV to induce a level of anesthesia sufficient for endotracheal intubation. The pig was maintained on a closed circuit system of halothane (3 to 7 percent; Ayerst Laboratories) and oxygen (300 to 500 cc per minute). The plane of surgical anesthesia was determined by the loss of the palpebral reflex, a heart rate between 80 and 120 after an initial increase above 150, and a respiration rate between 8 and 16.

Pituitary stalk-section was performed following a technique similar to that for hypophysectomy as described by du Mesnil du Buisson, Léglise and Chodkiewicz (1964). The anesthetized animal was restrained in an upright position on the operating table. The head was shaved, and was washed with green soap (Parke Davis and Co.) and with phisohex (Winthrop Laboratories). The head was then swabbed with 50 percent iso-propyl

alcohol and with 2 percent iodine. The head and neck, except for the area to be incised, and the instrument tables were covered with sterile towels. An incision was made through the skin to the frontal-parietal bone, beginning immediately above the lower third of the left eye and moving laterally 1 to 2 cm past the mid-line, vertically 8 to 9 cm toward the nuchal crest and laterally 7 to 8 cm just below the ear on the ipsilateral side. The skin and periosteum were freed from the frontal-parietal bone and folded to the side. The frontal-parietal bone was removed using a circular bone saw (Orthopedic Equipment Co., Inc.), osteotome and Rongeur Forceps to expose a 5 x 7 cm area of the left frontal lobe of the cerebral hemisphere and 0.5 to 1 cm of the right lobe along the sagittal sinus. Bleeding points in the bone and skin were cauterized and the bone was covered with bone wax. The exposed tissues were bathed with physiological saline containing 500,000 units of penicillin and 1 g of streptomycin sulfate per 500 ml. The dura mater was incised and an adjustable de Martel brain retractor (Collin, Gentile and Co.) was inserted, between the dura mater and arachnoid, to lift the cerebral hemisphere and expose the pituitary. The internal carotid artery was clamped with two silver clips and was severed between the clips. The pituitary stalk was severed using silver tipped glass probes. A nylon disc (8 mm diameter x 0.5 mm thickness) was placed between the severed ends of the stalk to prevent vascular regeneration to the anterior pituitary. A small (3 x 15 mm) piece of absorbable gelatin sponge (Gelfoam; Upjohn Co.) soaked in topical thrombin (Bovine, 1000 NIH units dissolved in 10 ml sterile water; Parke Davis and Co.) was placed near the pituitary to control hemorrhage. The retractor

was then removed. The pituitary stalk was checked 3 times at 3 to 5 minute intervals to insure that bleeding had stopped. A polyethylene film was placed over the arachnoid and the dura mater was sutured loosely. Gelfoam soaked in topical thrombin was placed on the dura mater. Powdered penicillin (500,000 units) was applied on the gelfoam. The frontal-parietal bone was replaced, covered with penicillin (1,000,000 units) and streptomycin sulfate (1 g), and the skin sutured. The pig was given 500 ml of 5 percent glucose solution IV and taken off anesthesia.

Three groups, each consisting of 9 pigs, were pituitary stalk-sectioned and subsequently ovariectomized as follows: A, on day 2 of the estrous cycle and ovariectomized on day 12, 16 or 20; B, on the day after the first day of mating (day 2) and ovariectomized on day 12, 16 or 20; and C, hysterectomized on days 5 to 7 of the estrous cycle, pituitary stalk-sectioned on days 20 to 22 and ovariectomized 4, 8 or 12 days later. Ovarian function in these pigs was compared with 4 animals ovariectomized on day 13 of the estrous cycle, 3 on day 13 of pregnancy and 3 hysterectomized pigs 24 to 26 days after estrus.

Eight pigs, were pituitary stalk-sectioned on the day after estrus or mating (day 2). Two cycling and 2 mated animals each received 2 daily IM injections of 2.5 mg of estradiol-17 β (estradiol-benzoate in corn oil; Nutritional Biochemicals) from day 12 to 20 and were ovariectomized on day 20. The other 4 pigs, 2 cycling and 2 mated, received 2 daily IM injections of a saline suspension of 25 mg of desiccated porcine anterior pituitary from day 12 to 20 and were ovariectomized on day 20. Ovarian function in these pigs was compared with 5 intact control

animals whose corpora lutea were marked with black silk suture on days 5 to 7. Two of these control pigs received the same level of estrogen from day 12 to 20. Three of the control animals received the same level of desiccated porcine anterior pituitary from day 10 to 22.

Hypophysectomy was performed following the same procedure as described for pituitary stalk-section except that the animal received an IM injection of 100 mg rather than 50 mg of cortisone acetate prior to surgery. The pituitary was removed using silver tipped glass probes and spatulae.

Four pigs were hypophysectomized on the day after estrus and were unilaterally ovariectomized on day 10 or 11. Four intact animals ovariectomized on day 13 of the cycle were used as controls.

Five animals were hysterectomized on days 5 to 7 and were hypophysectomized on days 20 to 22 after estrus. Three of these pigs received twice daily IM injections of 25 mg of a saline suspension of desiccated porcine anterior pituitary from the day of hypophysectomy to ovariectomy (days 32 to 34). Two animals received twice daily IM injections of 1000 IU of HCG (chorionic gonadotrophin; Nutritional Biochemicals) from the day of hypophysectomy to ovariectomy (day 33 or 34). Three pigs hysterectomized on days 5 to 7 and ovariectomized on day 20 were used as controls.

Seven pigs were hypophysectomized and treated as follows: A, one animal hypophysectomized on day 7 was ovariectomized on day 15; B, one pig hypophysectomized on day 2 was unilaterally ovariectomized on day 68, received 2 daily IM injections of 600 IU of PMSG (pregnant mare serum gonadotropin; Ayerst Laboratories) from day 72 to 76 and 1 IM injection

of 100 IU of HCG on day 78, and then ovariectomized on day 80; C, one animal hypophysectomized on day 2 and unilaterally ovariectomized on day 12 received 2 daily IM injections of 375 IU of HCG from days 12 to 20 and the remaining ovary was removed on day 20; D, one pig hypophysectomized on day 7 received 2 daily IM injections of 625 IU of HCG from days 10 to 25 and was unilaterally ovariectomized on days 17 and 25; E, two animals hypophysectomized on day 2 and unilaterally ovariectomized on day 12 received 2 daily IM injections of 6.25 mg of GH (growth hormone; Armour and Co.) from days 12 to 20 and were ovariectomized on day 20; and F, one pig hysterectomized on day 9 and hypophysectomized on day 22 received 2 daily IM injections of 12 mg of porcine prolactin (Upjohn Co.) from day 23 to 29 and was ovariectomized on day 29. The potency of the desiccated porcine anterior pituitary preparation was equivalent to 2.02 μ g of NIH-LH-S1 per mg of dry powder as determined by the ovarian ascorbic acid depletion assay (Parlow, 1961).

Following surgery the pig was placed in a room maintained at approximately 30°C. Rectal temperatures were recorded frequently during the first 8 to 10 hours after surgery and 1 to 3 times daily thereafter until the animal was sacrificed. The animals were fed about 300 g each of powdered milk and sugar and 5 g of salt (NaCl) dissolved in 3 to 4 l. of water 3 to 4 times during the first 10 hours after surgery. In addition hypophysectomized pigs were given IM injections of 25 to 75 mg of cortisone acetate and hydrochlorothiazide during the first 10 hours after surgery. Pituitary stalk-sectioned pigs usually required no post operative hormone therapy. About 10 l. of milk and sugar mixture

were given to the animals each day until they commenced normal food and water consumption. Following surgery, food and water consumption returned to normal 2 to 4 days later in hypophysectomized pigs and 1 to 2 days later in stalk-sectioned animals.

A description of the average rectal temperature changes during and following surgery is shown in figure 1.

The ovaries were placed on ice immediately after ovariectomy. The weight of the ovaries, and the number, size and condition of the follicles and corpora albicantia were recorded. The corpora lutea were dissected from the ovaries, weighed, and duplicate composite samples of about 500 mg of tissue were placed in 30 ml of 95 percent ethanol. At the beginning of ethanol extraction of the luteal tissue, $0.05 \mu\text{C}$ progesterone- 4C^{14} was added for estimation of procedural losses. Luteal tissue progesterone concentrations were corrected for the recovery rate from each sample. Progesterone in these tissue samples was analyzed as described by Masuda et al. (1966).

Uteri of mated pigs were examined for the presence of live embryos. The day 12 uteri were flushed with physiological saline to remove the blastocysts. The day 16 and 20 uteri were opened and submerged in physiological saline to free the gastrulae.

All pigs were sacrificed either at the termination of the experiment or 2 weeks after ovariectomy. The brain and pituitary gland were examined to insure that the nylon disc remained in position and that there was no vascular regeneration to the anterior pituitary. In the stalk-sectioned animals the pituitary was weighed and prepared for histological exam-

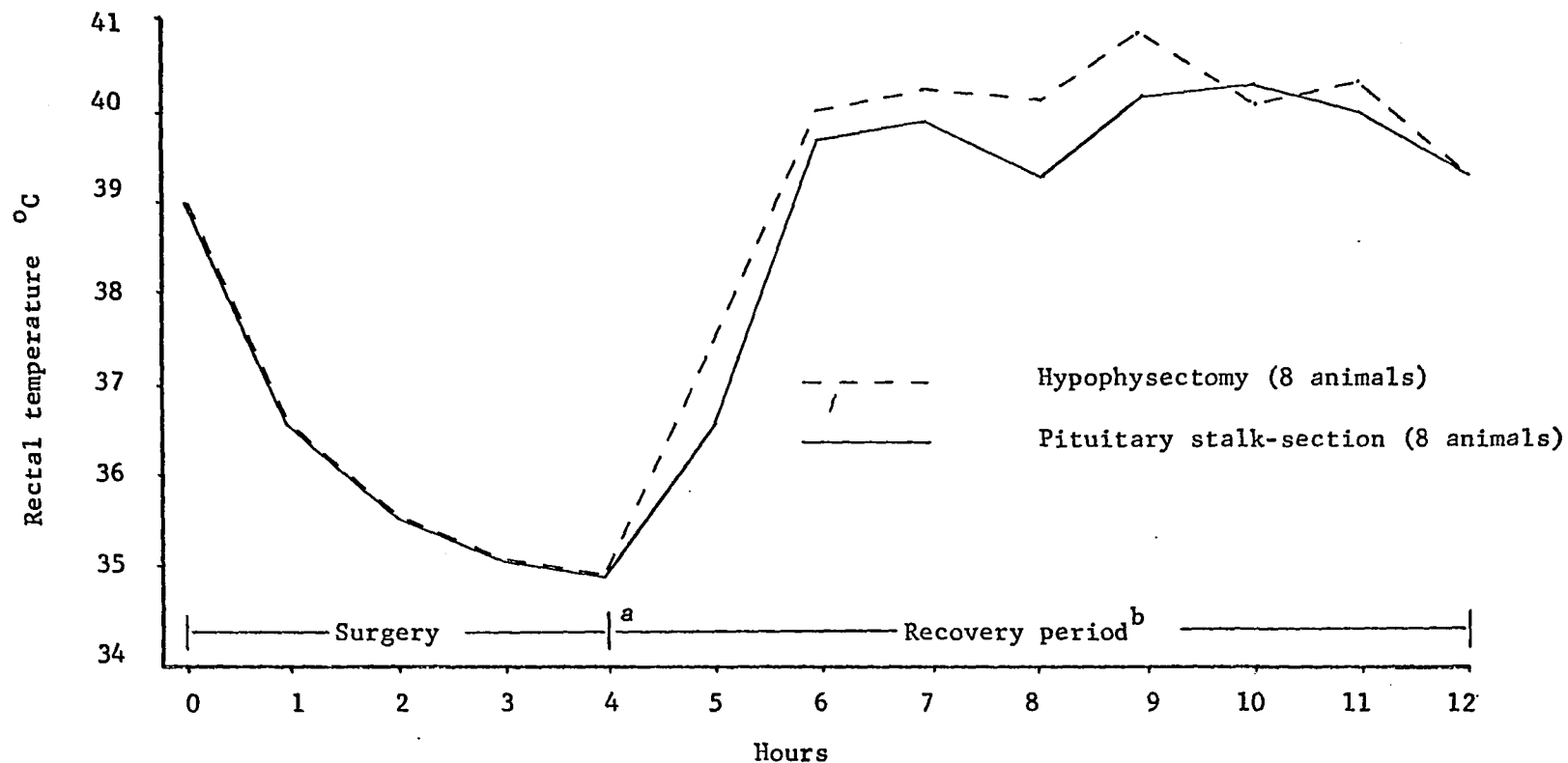


Figure 1. Average rectal temperature during surgery and the recovery period

^a500 ml of a 5 percent glucose solution IV.

^bAll animals received 3 to 4 l. of a milk and sugar mixture at 2-to 3-hour intervals. The hypophysectomized animals also received IM injections of 50 mg of cortisone acetate or hydrochlorothiazide at 2-to 3-hour intervals.

ination. The glands were fixed in Susa's fluid and stored in 70 percent ethanol. Sections were cut at 4 to 5 μ and stained with either; aldehyde-thionin, PAS (periodic-acid-Schiff reagent; basic fuchsin, sodium metabisulfite and hydrochloric acid), hematoxylin, orange G and aqueous phosphotungstic acid, or hematoxylin and eosin. The sella turcica in hypophysectomized pigs was examined for the presence of pituitary tissue.

Statistical analysis employed were the "t" test and orthogonal comparisons in regression as described by Snedecor (1956).

RESULTS

Functional corpora lutea developed in the ovaries of the pig following pituitary stalk-section performed on the day after estrus or mating (day 2) as shown in table 1. On day 12 the corpora lutea in cycling pituitary stalk-sectioned animals were significantly ($P < 0.05$) smaller than those in mated stalk-sectioned pigs. They also were smaller ($P < 0.05$) in cycling pituitary stalk-sectioned animals than in unoperated cycling or mated pigs at this time. Luteal tissue progesterone concentration was similar ($P > 0.05$) on the 12th day in both pituitary stalk-sectioned cycling and mated animals when compared to that found in unoperated cycling and mated pigs.

Sixteen days after estrus the progesterone concentration in the corpora lutea of the cycling pituitary stalk-sectioned pigs was significantly ($P < 0.01$) lower than in mated stalk-sectioned animals. Corpora lutea regression 20 days after estrus or mating in these stalk-sectioned pigs was similar to that found in intact animals at the same stage of the estrous cycle. Luteal regression began within 4 days and was complete by 12 days after pituitary stalk-section of previously hysterectomized pigs (table 1). Corpora lutea in cycling and mated pituitary stalk-sectioned animals showed significant ($P < 0.01$) linear regression in both mean corpus luteum weight and progesterone concentration after the 12th day. Linear regression ($P < 0.01$) of corpora lutea of previously hysterectomized pigs began within 4 days after pituitary stalk-section. A significant ($P < 0.01$) quadratic regression in mean corpus luteum weight and progesterone concen-

tration also was observed in cycling pituitary stalk-sectioned animals.

Graafian follicles larger than 3 mm diameter were absent in the ovaries 10 days after pituitary stalk-section of the cycling and mated pigs. Only atretic follicles were present 8 days after stalk-section of hysterectomized animals.

Blastocysts or gastrulae were present in the uteri of mated animals on days 12 and 16 and necrotic embryonic tissues were found on day 20.

Daily IM injections of estradiol-benzoate maintained the corpora lutea of intact cycling pigs, but not pituitary stalk-sectioned cycling or mated animals (table 2). One pig stalk-sectioned on the day after estrus had functional corpora lutea at the time of ovariectomy (day 20). Examination of the pituitary gland at the time of sacrifice revealed partial vascular regeneration. Observations on this animal are not included in table 2.

Desiccated porcine anterior pituitary injections maintained the corpora lutea and pregnancy for at least 20 days in pigs pituitary stalk-sectioned on the day after mating (table 2). Atretic follicles 1 to 3 mm diameter were present in the ovaries of these pigs. Two animals stalk-sectioned on the day after estrus and ovariectomized on day 20 had functional corpora lutea and excessive follicular growth similar to intact pigs receiving desiccated porcine anterior pituitary injections. Partial vascular regeneration of the pituitary was observed when these 2 animals were sacrificed. Observations of these 2 pigs are not included in the table. The daily IM injection of larger quantities of desiccated porcine anterior pituitary in intact animals resulted in excessive luteal and follicular development, while lower levels produced variable results (Appendix, table 9).

Table 1. Ovarian function in pituitary stalk-sectioned pigs

Reproductive stage	Days after		No. of pigs	Ovarian wt. ^a (g)	Mean no.	Corpora lutea		
	Estrus	Pituitary stalk-section				Mean wt. ^a (mg)	Progesterone Total ^a (µg)	Conc. (µg/g) ^a
Estrous cycle	13	Intact control	4	12.3±0.7	9.5	490±39	317±25	69±3
	12	10	3	7.9±0.4	13.7	305±32	262±22	64±4
	16	14	3	8.4±0.4	13.7	260±16	8± 6	3±2
	20	18	3	4.3±0.9	14.3	52±12	3± 1	5±1
Pregnancy ^b	13	Intact control	3	11.7±1.5	9.3	466±32	268±42	65±2
	12	10	3	10.2±1.4	10.0	431±16	239±10	57±6
	16	14	3	7.2±0.9	11.3	292±46	94±20	28±4
	20	18	3	6.1±0.4	12.3	133±52	12± 5	7±1
Hysterectomy ^c	24-26	Control	3	13.1±0.8	11.0	392±18	309±32	64±1
	24-26	4	3	8.7±1.3	13.0	187±13	74±20	31±9
	28-30	8	3	5.7±0.7	12.0	93±15	24± 3	25±8
	32-34	12	3	5.0±0.8	11.7	47± 1	8± 4	13±4

^aMean ± SE.

^bAn average of 8 live blastocysts or gastrulae (day 12), 10 live gastrulae (day 16) and 20 (day 20) were present in the uteri of the pituitary stalk-sectioned pigs.

^cThe animals were hysterectomized on days 5 to 7 after estrus.

d pigs

Mean no.	Corpora lutea		Avg. no. of Graafian follicles			Condition	
	Mean wt. ^a (mg)	Progesterone	Follicle diameter				
		Total (μ g) ^a	Conc. ^a (μ g/g)	(mm)			
				1-3	4-6	7-9	
9.5	490+39	317+25	69+3	4	12	1	normal
13.7	305+32	262+22	64+4	17	0	0	atretic
13.7	260+16	8+ 6	3+2	22	0	0	atretic
14.3	52+12	3+ 1	5+1	21	0	0	atretic
9.3	466+32	268+42	65+2	-	12	11	normal
10.0	431+16	239+10	57+6	56	0	0	atretic
11.3	292+46	94+20	28+4	11	0	0	atretic
12.3	133+52	12+ 5	7+1	57	0	0	atretic
11.0	392+18	309+32	64+1	17	21	0	normal
13.0	187+13	74+20	31+9	12	14	0	normal
12.0	93+15	24+ 3	25+8	20	10	0	atretic
11.7	47+ 1	8+ 4	13+4	39	5	0	atretic

53

12), 10 live gastrulae (day 16) and 11 regressed gastrulae
c-sectioned pigs.

er estrus.

Table 2. Ovarian function in pituitary stalk-sectioned pigs after the injection of estr anterior pituitary

Reproductive stage	Days after Pituitary stalk-section		No. of pigs	Ovarian wt. (g) ^a	Mean no.	Corpora lutea		
						Mean wt. (mg) ^a	Total Progesterone (μg) ^a	Conc. (μg/g)
Animals received twice daily IM injections of the equivalent of 2.5 mg es								
Estrous cycle	20	Intact control	2	12.7 \pm 1.1	12.0	393 \pm 12	284 \pm 22	60 \pm
	20	18	1	5.9	12	148	11	6
Pregnancy	20	18	2 ^b	4.6 \pm 0.4	11.5	85 \pm 17	4 \pm 3	5 \pm
Animals received twice daily IM injection of 25 mg of desiccated porcine								
20.								
Estrous cycle	22	Intact control	3	180 \pm 79	28.7	631 \pm 119	993 \pm 329	54 \pm
Pregnancy	20	18	2 ^c	9.4 \pm 0.1	14.0	464 \pm 6	408 \pm 60	63 \pm 10

^aMean ± SE.

^bThe uteri of these pigs contained 8 and 9 regressed embryonic membranes on day 20.

^cThe uteri of these pigs contained 9 and 10 live embryos on day 20.

ary stalk-sectioned pigs after the injection of estradiol-benzoate or desiccated porcine

No. of pigs	Ovarian wt. (g) ^a	<u>Corpora lutea</u>				<u>Avg. no. of Graafian follicles</u>				Condition
		Mean no.	Mean wt. (mg) ^a	<u>Progesterone</u>		<u>Follicle diameter</u>				
				Total (μg) ^a	Conc. (μg/g) ^a	(mm) —				
						1-3	4-6	7-15	16-40	

e daily IM injections of the equivalent of 2.5 mg estradiol-17 β from day 12 to 20.

2	12.7 \pm 1.1	12.0	393 \pm 12	284 \pm 22	60 \pm 7	13	39	0	0	normal
1	5.9	12	148	11	6	73	0	0	0	atretic
2 ^b	4.6 \pm 0.4	11.5	85 \pm 17	4 \pm 3	5 \pm 5	25	0	0	0	atretic

e daily IM injection of 25 mg of desiccated porcine anterior pituitary from day 12 to

3	180 \pm 79	28.7	631 \pm 119	993 \pm 329	54 \pm 3	-	15	19	14	cystic
2 ^c	9.4 \pm 0.1	14.0	464 \pm 6	408 \pm 60	63 \pm 10	6	0	0	0	atretic

ed 8 and 9 regressed embryonic membranes on day 20.

ed 9 and 10 live embryos on day 20.

The effect of hypophysectomy on ovarian function during the estrous cycle is shown in table 3. Functional corpora lutea were present in the ovaries of animals hypophysectomized on day 2 and ovariectomized on day 10 or 11 after estrus. The corpora lutea, however, were significantly ($P < 0.01$) smaller than those of control pigs ovariectomized on day 13 of the estrous cycle but contained similar progesterone concentrations.

HCG or desiccated porcine anterior pituitary injections maintained luteal function in hypophysectomized-hysterectomized animals (table 3). Corpora lutea and progesterone concentration were maintained in 3 hysterectomized controls. Graafian follicles were completely regressed in the ovaries of the hypophysectomized pigs. Atretic follicles 1 to 3 mm diameter were present in the ovaries of those hypophysectomized animals receiving injections of desiccated porcine anterior pituitary. Corpora lutea of animals receiving the pituitary injections were significantly ($P < 0.05$) larger and contained more ($P < 0.01$) progesterone than the hysterectomized control pigs.

One animal hypophysectomized on day 7 was ovariectomized on day 15. The ovaries contained 9 corpora lutea with an average weight of 309 mg and a progesterone concentration of 26 $\mu\text{g/g}$.

One pig hypophysectomized on day 2 was unilaterally ovariectomized on day 68. The ovary was regressed and weighed 0.4 g. An attempt to stimulate follicular growth and ovulation in the remaining ovary with twice daily IM injections of 600 IU of PMSG for 3 days, followed by a single injection of 100 IU of HCG did not change the condition of the ovary. This ovary weighed 0.26 g on day 83.

Table 3. Ovarian function in pigs hypophysectomized on the day after estrus or after hypophysectomy and gonadotropic hormone treatment

Reproductive stage	<u>Day of estrous cycle for</u> Hypophysectomy Ovariectomy		No. of pigs	Ovarian wt. (g) ^a	Mean no.	Mean wt. (mg) ^a	Prog. Total (μg)
Estrous cycle	Intact control	13	4 ^b	6.2±0.7	4.5	466±19	145±19
	2	10-11	4 ^b	4.9±0.7	5.8	257±44	105±19
Hysterectomy ^c	None	19-21	3	11.8±0.1	10.0	430±36	299±19
Animals received twice daily IM injections of 25 mg of desiccated porcine							
	20-22	32-34	3	11.3±1.2	10.0	606±52	490±19
Animals received twice daily IM injections of 1000 IU of HCG from day 21 to 22							
	21-22	33-34	2	10.7±1.5	11.5	350±199	238±19

^a Mean ± SE.

^b One ovary per animal.

^c The pigs were hysterectomized on days 5 to 7 after estrus.

hysterectomized on the day after estrus or after hysterectomy and the effect of

No. of pigs	Ovarian wt. (g) ^a	Mean no.	Mean wt. (mg) ^a	<u>Progesterone</u>		<u>Graafian follicles</u> <u>Follicle diameter</u> (mm)			Condition
				Total (μg) ^a	Conc. (μg/g) ^a	1-3	4-6	7-9	
4 ^b	6.2±0.7	4.5	466±19	145±16	69±3	-	14	2	normal
4 ^b	4.9±0.7	5.8	257±44	105±35	67±5	-	0	0	regressed
3	11.8±0.1	10.0	430±36	299±34	70±4	-	13	1	normal
ly IM injections of 25 mg of desiccated porcine anterior pituitary from day 20 to 34.									
3	11.3±1.2	10.0	606±52	490±19	84±12	25	0	0	atretic
ly IM injections of 1000 IU of HCG from day 21 to 34									
2	10.7±1.5	11.5	350±199	238±105	66±4	0	0	0	regressed

ys 5 to 7 after estrus.

Twice daily IM injections of either 375 or 625 IU of HCG did not maintain functional corpora lutea in the ovaries of hypophysectomized animals. One pig receiving the lower level of HCG was unilaterally ovariectomized on day 21. The ovary weighed 1.9 g and contained 8 regressed corpora lutea with an average weight of 21 mg. One animal received 625 IU of HCG twice daily and was unilaterally ovariectomized on days 17 and 25. The ovary removed on day 17 weighed 4.0 g and contained 5 corpora lutea with an average weight of 341 mg and a progesterone concentration of 10 $\mu\text{g/g}$. The ovary removed on day 25 weighed 3.1 g and contained 6 corpora lutea with an average weight of 109 mg and a progesterone concentration of 14 $\mu\text{g/g}$.

Two daily IM injections of 6.25 mg of growth hormone from day 12 to 20 in 2 hypophysectomized pigs did not maintain luteal function at the time of unilateral ovariectomy on day 20. The ovaries weighed 2.0 ± 0.7 g and contained 7 ± 2 corpora lutea with an average weight of 70 ± 22 mg and a progesterone concentration of less than 2 $\mu\text{g/g}$. The growth hormone, however, produced marked edema of the endometrium.

Porcine prolactin given in 2 daily IM injections of 12 mg did not maintain luteal function in one hypophysectomized-hysterectomized pig. The ovaries weighed 4.5 g and contained 10 corpora lutea 6 days after hypophysectomy. The corpora lutea weighed an average of 189 mg and contained 23 μg progesterone per gram of tissue.

The mean weight of pituitaries from the 35 stalk-sectioned pigs was 143 ± 9 mg at the time of sacrifice, whereas from 5 intact pigs the weight was 318 ± 19 mg. No differences in pituitary size were observed with time after stalk-section (14 to 35 days). In the anterior pituitaries of intact

pigs 6 cell types were identified; orange to yellow acidophils, red to brown acidophils, PAS positive aldehyde-thionin positive cells, PAS positive aldehyde-thionin negative cells (faint staining cells and large deep staining cells), PAS negative aldehyde-thionin positive cells and chromophobes. These same cell types also were identified in the anterior pituitaries from stalk-sectioned pigs. The affinity for the stains, however, was reduced and the cell types were difficult to identify. Numerous blood vessels were found in both intact and stalk-sectioned anterior pituitaries. The stalk-sectioned pituitaries were more deeply stained with eosin than were the intact pituitaries.

DISCUSSION

Corpora lutea developed in the ovaries of pituitary stalk-sectioned pigs during the period of 12 days after estrus or mating. In animals stalk-sectioned the day after estrus, the corpora lutea were smaller than found in the mated pituitary stalk-sectioned, estrous cycle control or mated control pigs by day 12. Corpora lutea of the cycling stalk-sectioned animals at day 16 contained less progesterone than the mated pituitary stalk-sectioned pigs. These corpora lutea were smaller than found on day 12, but there was no difference in mean luteal weight between the cycling and mated stalk-sectioned pigs at this time. Twenty days after estrus or mating the corpora lutea of stalk-sectioned pigs contained low concentrations of progesterone.

Development of corpora lutea during the first 10 days after pituitary stalk-section was similar to that of hypophysectomy, as shown in table 3, and similar to that previously reported by du Mesnil du Buisson and Léglise (1963). Progesterone injections commencing at estrus (Brinkley et al., 1964) or after mating (Sammelwitz et al., 1961) did not interfere with the development of the corpora lutea for 12 days. Progesterone given beyond days 12 to 16 induced rapid destruction of the corpora lutea in mated pigs.

The decline in luteal progesterone concentration observed in both mated and hysterectomized pituitary stalk-sectioned pigs was similar to that found during the last third of the estrous cycle (Masuda et al., 1966). This indicates that the intact pituitary secreted luteotropic hormone, but not in sufficient amounts to override uterine luteolytic action.

In stalk-sectioned pigs, a uterine luteolytic effect limited progesterone production after day 12 and before day 16 in the cycle, whereas the presence of embryos at day 16 inhibited luteolytic action but progesterone production was not maintained. Lower progesterone concentration in the corpora lutea of the mated pigs suggested that there was a reduction in luteotropin secretion from the stalk-sectioned pituitary; necrotic embryonic tissue was present in the uteri on day 20.

Failure of the stalk-sectioned pituitary to maintain the corpora lutea in hysterectomized pigs indicated that secretion of luteotropic hormone was dependent upon hypothalamic stimulation. Corpora lutea persisted, though progesterone content was low, for 20 days in 4 pigs that were stalk-sectioned after the 70th day of gestation or hysterectomy (du Mesnil du Buisson, 1966). Corpora lutea were maintained at least 15 days after pituitary stalk-section of hysterectomized sheep (Denamur et al., 1966). Hypophysectomy after estrus resulted in a gradual decline in luteal function while the corpora lutea were maintained until day 15 of the estrous cycle in pituitary stalk-sectioned sheep. Hypophysectomy of hysterectomized sheep resulted in immediate regression of the corpora lutea.

The failure of exogenous estrogen to maintain luteal function after pituitary stalk-section indicated that it may cause the hypothalamus to induce pituitary luteotropin release in this species. Corpora lutea and luteal progesterone production were maintained by daily injections of the same level of estrogen in intact control pigs. Gardner et al. (1963) reported luteal maintenance by exogenous estrogen in intact pigs.

Desiccated porcine anterior pituitary acted directly by maintaining luteal progesterone production during the first 20 days of pregnancy in stalk-sectioned pigs. du Mesnil du Buisson et al. (1964) reported that pregnancy was maintained in hypophysectomized pigs by daily injections of progesterone and estrogen for the first quarter and with progesterone alone for the duration of gestation. The corpora lutea regressed in these animals.

Hypophysectomy on the day after estrus did not interfere with the development of corpora lutea to day 10 or 11. These corpora lutea were smaller than those found in intact control animals. Similar results were reported by du Mesnil du Buisson and Léglise (1963). Corpora lutea regressed by 20 days after estrus in these hypophysectomized animals.

Maintenance of corpora lutea after hypophysectomy of hysterectomized animals was obtained with daily IM injections of either desiccated porcine anterior pituitary or HCG beginning at the time of hypophysectomy. HCG maintained the corpora lutea in a condition similar to that of hysterectomized control pigs, whereas desiccated porcine anterior pituitary produced a significant ($P < 0.05$) increase in luteal weight as well as total progesterone content. Anderson et al. (1965) reported luteal maintenance in hypophysectomized-hysterectomized pigs with injections of either desiccated ovine pituitary, HCG or LH (bovine or equine). These same treatments were ineffective in maintaining luteal function in hypophysectomized pigs with intact uteri. The investigations reported here and the results of Anderson et al. (1965) suggest that the main-

tenance of the corpora lutea following hypophysectomy is dependent upon exogenous gonadotropin as well as the absence of the uterus.

Normal growth of follicles was absent in pituitary stalk-sectioned pigs and no follicular growth in hypophysectomized pigs. Atretic follicles in stalk-sectioned pigs indicated that there was residual gonadotropin secretion which was not sufficient to maintain them. Small follicles found in the ovaries of hypophysectomized pigs receiving desiccated porcine anterior pituitary were attributed to the exogenous gonadotropins.

Though the anterior pituitaries were smaller in stalk-sectioned pigs, histological examination showed the presence of blood vessels throughout the anterior pituitary and there was an absence of necrotic tissue. The glandular cells in anterior pituitaries from stalk-sectioned animals had less affinity for the stains than did the cells in the intact pituitaries. This difference suggests that the anterior pituitary separated from the hypothalamus did not produce or secrete appreciable amounts of the hormones by 14 to 35 days after stalk-section. Daniel and Prichard (1957) and Cowie et al. (1963) reported extensive anterior pituitary necrosis following stalk-section of the goat and sheep.

SUMMARY

This investigation deals with the effect of pituitary stalk-section or hypophysectomy on ovarian function in the pig. Pituitary stalk-section was performed following estrus, mating or hysterectomy. Hypophysectomy was performed during the luteal phase of the estrous cycle or after hysterectomy. The effect of exogenous hormones on ovarian function following pituitary stalk-section or hypophysectomy also was investigated.

Corpora lutea developed in pigs pituitary stalk-sectioned the day after estrus or mating. Twelve days after estrus corpora lutea in cycling pituitary stalk-sectioned animals were smaller than those in either mated stalk-sectioned, estrous cycle control or mated control pigs. Luteal progesterone concentration 16 days after estrus was 3 $\mu\text{g/g}$ in cycling stalk-sectioned animals and 28 $\mu\text{g/g}$ in mated stalk-sectioned pigs. Twenty days after estrus the corpora lutea and luteal progesterone concentration in both cycling and mated stalk-sectioned animals was similar to that found on day 20 of the cycle.

Blastocysts or gastrulae were present in the uteri of these stalk-sectioned animals 12 and 16 days after mating and only necrotic embryos were found at day 20.

Uterine luteolytic action appeared to terminate progesterone production by day 16 in the cycling pituitary stalk-sectioned pigs. Living embryos inhibited the uterine luteolytic effect at this time.

Pituitary stalk-section of hysterectomized pigs resulted in regressive changes in the corpora lutea within 4 days. By 12 days the corpora

lutea had regressed and the progesterone concentration had declined.

Failure of the corpora lutea in mated, and hysterectomized pituitary stalk-sectioned pigs indicated that hypothalamic stimulation of the pituitary was necessary for luteotropic hormone secretion.

Estradiol-benzoate maintained corpora lutea in intact pigs but not in animals pituitary stalk-sectioned on the day after estrus or mating. Pregnancy was not maintained by estrogen treatment in these animals.

Desiccated porcine anterior pituitary maintained corpora lutea in intact and pituitary stalk-sectioned pigs. Pregnancy was also maintained in these stalk-sectioned animals. This treatment caused excessive follicular growth and accessory corpora lutea formation in intact pigs, but not in stalk-sectioned animals. Desiccated porcine anterior pituitary or HCG maintained corpora lutea in hypophysectomized pigs that were previously hysterectomized.

Only atretic follicles were present in the ovaries of pituitary stalk-sectioned pigs. All follicles had regressed in the ovaries of hypophysectomized pigs. Follicular development in the pig is dependent upon hypothalamic stimulation of the pituitary gland.

The anterior pituitary was smaller than found in intact animals, but remained viable 14 to 35 days after pituitary stalk-section. Histological examination of the stalk-sectioned glands indicated that gonadotropic hormone production by the glandular cells was reduced by this time.

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APPENDIX

Table 4. Ovarian function in pituitary stalk-sectioned pigs during the estrous cycle

Days after Estrus	Pituitary stalk-section ^a	Ovarian wt. (g)	Corpora lutea			No. of Graafian follicles			Condition
			No.	Mean Progesterone		Follicle diameter			
				wt. (mg)	conc. (µg/g)	(mm)			
						1-3	4-6	7-9	
13	Intact control	13.6	8	474	78	4	6	2	normal
		11.3	10	500	67	2	16	0	normal
		12.7	10	558	64	5	15	0	normal
		<u>11.7</u>	<u>10</u>	<u>426</u>	<u>66</u>	<u>5</u>	<u>11</u>	<u>3</u>	normal
Average		<u>12.3</u>	<u>9.5</u>	<u>490</u>	<u>68.7</u>	<u>4</u>	<u>12</u>	<u>1</u>	
12	10	7.5	13	282	65	18	0	0	atretic
		8.6	16	264	58	0	0	0	atretic
		<u>7.7</u>	<u>12</u>	<u>369</u>	<u>70</u>	<u>33</u>	<u>0</u>	<u>0</u>	atretic
		<u>7.9</u>	<u>13.7</u>	<u>305</u>	<u>64.3</u>	<u>17</u>	<u>0</u>	<u>0</u>	
Average									
16	14	8.9	16	228	2	18	0	0	atretic
		8.8	14	277	0	18	0	0	atretic
		<u>7.4</u>	<u>11</u>	<u>275</u>	<u>6</u>	<u>30</u>	<u>0</u>	<u>0</u>	atretic
		<u>8.4</u>	<u>13.7</u>	<u>260</u>	<u>2.7</u>	<u>22</u>	<u>0</u>	<u>0</u>	
Average									
20	18	6.0	15	74	4	33	0	0	atretic
		3.9	12	52	7	0	0	0	-
		<u>3.0</u>	<u>16</u>	<u>30</u>	<u>5</u>	<u>30</u>	<u>0</u>	<u>0</u>	atretic
		<u>4.3</u>	<u>14.3</u>	<u>52</u>	<u>5.3</u>	<u>21</u>	<u>0</u>	<u>0</u>	
Average									

^aThe pigs were pituitary stalk-sectioned on day 2 of the estrous cycle.

Table 5. Ovarian function in pituitary stalk-sectioned pigs during early pregnancy

Days after Pituitary Estrus stalk-section	Ovarian wt. (g)	Corpora lutea		No. of Graafian follicles				Embryos			
		No.	Mean Progesterone		Follicle diameter						
			wt. (mg)	conc. (µg/g)	(mm)			Condition	No.	Condition	
1 - 3	4 - 6	7 - 9									
13	Intact control	11.7	12	402	60	-	10	12	normal	-	-
		9.0	5	502	75	-	15	10	normal	-	-
		14.4	11	495	60	-	11	10	normal	-	-
Average		11.7	9.3	466	65.0		12	11			
12	10	7.7	9	401	62	56	0	0	atretic	9	blastocysts
		10.7	12	437	46	18	0	0	atretic	6	blastocysts
		12.4	9	456	63	95	0	0	atretic	8	gastrulae
Average		10.2	10.0	431	57.0	56	0	0		8	
16	14	6.1	10	271	34	0	0	0	-	10	gastrulae
		9.1	11	386	31	33	0	0	atretic	9	gastrulae
		6.4	13	230	20	0	0	0	-	12	gastrulae
Average		7.2	11.3	292	28.3	11	0	0		10	
20	18	6.6	14	37	6	95	0	0	atretic	11	regressed
		6.3	10	218	9	50	0	0	atretic	9	regressed
		5.4	13	143	7	25	0	0	atretic	12	regressed
Average		6.1	12.3	133	7.3	57	0	0		11	

^aThe pigs were pituitary stalk-sectioned on the day after the first day of mating.

Table 6. Ovarian function in hysterectomized pigs after pituitary stalk-section

Days after Pituitary ^a Estrus stalk-section		Ovarian wt. (g)	Corpora lutea			No. of Graafian follicles		Condition
			No.	Mean Progesterone		Follicle diameter		
				wt. (mg)	conc. (µg/g)	(mm)		
						1-3	4-6	
24-26	Control	14.8	12	422	66	30	10	normal
		12.4	11	361	62	0	24	normal
		<u>12.2</u>	<u>10</u>	<u>392</u>	<u>65</u>	<u>20</u>	<u>30</u>	normal
Average		13.1	11	392	64.3	17	21	
24-26	4	11.0	14	171	36	18	15	normal
		6.3	13	178	44	17	0	normal
		<u>8.7</u>	<u>12</u>	<u>212</u>	<u>14</u>	<u>0</u>	<u>28</u>	normal
Average		8.7	13	187	31.3	12	14	
28-30	8	7.1	14	123	11	27	7	atretic
		4.9	12	79	28	33	0	atretic
		<u>5.1</u>	<u>10</u>	<u>76</u>	<u>37</u>	<u>0</u>	<u>23</u>	atretic
Average		5.7	12	93	25.3	20	10	
32-34	12	4.3	10	45	--	25	0	atretic
		4.1	15	48	17	18	0	atretic
		<u>6.7</u>	<u>10</u>	<u>47</u>	<u>9</u>	<u>75</u>	<u>15</u>	atretic
Average		5.0	11.7	47	13.0	39	5	

^aThe pigs were hysterectomized on days 5 to 7 and pituitary stalk-sectioned on days 20 to 22 of the estrous cycle.

Table 7. Ovarian function in pituitary stalk-sectioned pigs after the injection of estradiol-benzoate^a

Days after		Ovarian wt. (g)	Corpora lutea			No. of Graafian follicles			Embryos	
Estrus stalk-section	Pituitary _b		Mean Progesterone		Follicle diameter	Follicle diameter		Condition		
		No.	wt. (mg)	conc. (µg/g)		(mm)			No.	Condition
						1-3	4-6			
20	Intact control	13.8	12	404	54	26	48	normal		
		<u>11.6</u>	<u>12</u>	<u>381</u>	<u>67</u>	<u>0</u>	<u>30</u>	normal		
Average		12.7	12	393	60.5	13	39			
20	18	5.9	12	148	6	73	0	atretic		
		<u>4.6</u>	<u>12</u>	<u>201</u>	<u>48</u>	<u>40</u>	<u>0</u>	atretic		
Average		5.2	12	174	27.0	56	0			
20	18	5.0	12	102	0	35	0	atretic	9	regressed
		<u>4.2</u>	<u>11</u>	<u>68</u>	<u>10</u>	<u>15</u>	<u>0</u>	atretic	8	regressed
Average		4.6	11.5	85	5.0	25	0			

^aThe pigs received twice daily IM injections of the equivalent of 2.5 mg estradiol-17 from day 12 to 20.

^bThe pigs were pituitary stalk-sectioned on day 2 of the estrous cycle or the day after the first day of mating.

Table 8. Ovarian function in pituitary stalk-sectioned pigs after the injection of desiccated porcine anterior pituitary^a

Days after		Ovarian wt. (g)	Corpora lutea			No. of Graafian follicles					Embryos	
Pituitary ^b Estrus stalk-section	No.		Mean Progesterone		Follicle diameter (mm)	Condition	No.	Condition				
			wt. (mg)	conc. (µg/g)								
						1-3	4-6	7-15	16-40			
22	Intact control	66	46 ^c	612	54	-	14	13	6	cystic		
		333	21 ^c	434	52	-	10	20	22		cystic	
		138	19 ^c	846	56	-	22	25	4		cystic	
		<u>180</u>	<u>28.7</u>	<u>631</u>	<u>54.0</u>		<u>15</u>	<u>19</u>	<u>14</u>			
Average												
20	18	39	50 ^c	236	40	-	25	38	0	cystic		
		64	28 ^c	335	38	-	36	15	0		cystic	
		<u>52</u>	<u>39.0</u>	<u>286</u>	<u>39.0</u>		<u>30</u>	<u>26</u>	<u>0</u>			
Average												
20	18	9.3	14	470	53	12	0	0	0	atretic regressed	9 gastrulae	
		9.4	14	458	73	0	0	0	0		10 gastrulae	
		<u>9.4</u>	<u>14.0</u>	<u>464</u>	<u>63.0</u>	<u>6</u>	<u>0</u>	<u>0</u>	<u>0</u>			
Average												

^aThe pigs received twice daily IM injections of 25 mg of desiccated porcine anterior pituitary from day 12 to 20.

^bThe pigs were pituitary stalk-sectioned on day 2 of the estrous cycle or the day after the first day of mating.

^cAccessory corpora lutea were present in the ovaries of these pigs.

Table 9. Ovarian function in intact pigs receiving twice daily intramuscular injections of desiccated porcine anterior pituitary

		Ovarian wt. (g)	Corpora lutea						No. of Graafian follicles					Condition
Pituitary powder			Marked ^a		Accessory		Progesterone conc.		Follicle diameter					
Per day			Mean wt.		Mean wt.		Marked	Accessory	(mm)					
(mg)	Days		No.	(mg)	No.	(mg)			1-3	4-6	7-15	16-40		
400	10-20	193	-	-	142 ^b	525	-	39	-	43	51	0	cystic	
100	10-22	173	10	881	71	658	32	49	-	35	59	3	cystic	
50	10-22	69 ^c	14	520	32	653	53	54	-	14	13	6	cystic	
		333	10	547	11	331	28	60	-	10	20	22	cystic	
		138	8	862	11	834	66	50	-	22	25	4	cystic	
Average		180	11	643	18	606	49	54.7		19	19	11		
25	10-22	12	9	147	0	0	12	0	-	12	51	0	normal	
		65	12	612	4	418	31	33	-	44	2	6	cystic	
10	10-22 ^d	10	15	105	0	0	-	0	-	33	12	0	normal	

^aThe corpora lutea were marked with black silk suture on days 5 to 7 after estrus.

^bThe corpora lutea in this pig were not marked prior to treatment.

^cThree large (16 to 40 mm) cystic follicles were broken during ovariectomy.

^dThis pig was in estrus on day 21.

Table 10. Ovarian function in pigs after hypophysectomy

Day of estrous cycle for		Ovarian wt. ^a (g)	Corpora lutea			No. of Graafian follicles			Condition
			No.	Mean Progesterone		Follicle diameter			
Hypophysectomy	Ovariectomy				wt. (mg)	conc. (µg/g)		(mm)	
						1-3	4-6	7-9	
Intact control	13	8.2	5	458	78	-	4	6	normal
		5.8	5	484	67	-	18	0	normal
		5.7	4	506	64	-	20	0	normal
		<u>5.2</u>	<u>4</u>	<u>416</u>	<u>66</u>	-	<u>13</u>	<u>2</u>	normal
Average		6.2	4.5	466	68.8		14	2	
2	10-11	3.1	4	288	57	0	0	0	regressed
		5.1	6	167	70	0	0	0	regressed
		6.5	6	206	62	0	0	0	regressed
		<u>4.9</u>	<u>7</u>	<u>366</u>	<u>78</u>	<u>0</u>	<u>0</u>	<u>0</u>	regressed
Average		4.9	5.8	257	66.8	0	0	0	

^a One ovary per animal.

Table 11. Effect of gonadotropins on ovarian function in hypophysectomized-hysterectomized pigs

Day of estrous cycle for			Ovarian wt. (g)	No.	Corpora lutea		No. of Graafian follicles			
Hysterectomy	Hypophysectomy	Ovariectomy			Mean Progesterone		Follicle diameter			Condition
					wt. (mg)	conc. (µg/g)	(mm)			
							1-3	4-6	7-9	
5-7	none ^a	19-21	11.7	8	502	69	-	17	1	normal
			11.8	10	399	64	-	13	3	normal
			11.9	12	390	78	-	19	0	normal
Average			11.8	10	430	70.3		13	1	
5-7	20-22 ^b	32-34	13.7	10	710	67	20	0	0	atretic
			8.5	8	552	106	30	0	0	atretic
			11.7	12	556	79	25	0	0	atretic
Average			11.3	10	606	84.0	25	0	0	
6-7	21-22 ^c	33-34	9.2	9	549	70	0	0	0	regressed
			12.2	14	152	62	0	0	0	regressed
Average			10.7	11.5	350	66.0	0	0	0	

^aNo hormone treatment.

^bThese pigs received twice daily IM injections of desiccated porcine anterior pituitary from day 20 to 34.

^cThese pigs received twice daily IM injections of 1000 IU of HCG from day 21 to 34.